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Combat behavior in *Centrolene buckleyi* and other centrolenid frogs

Wilmar BOLÍVAR-G., Taran GRANT [1] & Luis A. OSORIO

Laboratorio de Herpetología, Departamento de Biología, Universidad del Valle, A. A. 25360, Cali, Valle del Cauca, Colombia

Observations of fighting behavior in *Centrolene buckleyi* revealed that males dangled by their feet and grappled venter-to-venter. One of the males repeatedly uttered a soft, short squeak, inflating the vocal sac and prying off the other combatant in the process. Sonagrams of this aggressive call and the advertisement call reveal markedly different structures. Both frogs were visibly injured, presumably in combat. Of the few species coded for combat behavior ($n = 7$), those of the genera *Centrolene* and *Cochranella* exhibit the derived state of dangling by the feet and grappling venter-to-venter, whereas species of *Hyalinobatrachium* have primitive combat composed of one male grasping the other in amplexus. We predict that the derived behavior will be discovered in all *Centrolene* and in all or a large part of *Cochranella* (representing a synapomorphy that unites the two groups), and that no *Hyalinobatrachium* species will exhibit the apomorphic state.

INTRODUCTION

Although the past few decades have seen an unprecedented increase in our knowledge of centrolenid frogs, most workers have concentrated on resolving taxonomic and phylogenetic issues from a strictly morphological perspective. In so doing, they have inadvertently ignored an abundance of characters that could provide invaluable clues as to the relationships of these frogs. The purposes of this paper are to describe the physical combat of *Centrolene buckleyi* (Boulenger, 1882) and to discuss the phylogenetic significance of combat behavior in the family Centrolenidae.

1. Current address: Department of Herpetology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA.

Methods

Our observations of *Centrolene buckleyi* combat were made on 2 April 1996, just below El Boquerón, near the border between Departamento del Valle del Cauca and Departamento del Chocó at 2220 m in the Cordillera Occidental of Colombia (GPS coordinates: 4°44′39″N, 76°18′16″W). The site is in relatively intact cloud forest along the road from El Cairo to El Boquerón.

The frogs were found at the side of the road approximately 2.5 m directly above a trickle of run-off water and 6 m from a fast flowing stream along which we heard many advertisement calls of this species. It rained quite heavily throughout most of the day and night, before, during and after these observations.

Calls were recorded using a Sony WM D6C Professional Walkman and a Sony ECM 909 microphone at 15.6°C air temperature. Sonagrams were generated on a Kay 5500 DSP Sona-Graph. Data were also obtained using Computerized Speech Research Environment (CSRE) 4.5 PC-based signal analysis software.

Preserved specimens are kept in the Universidad del Valle Colección de Anfibios y Reptiles (UVC).

Results

Our observations began at 22.05 h. Frog A (UVC 12729; SVL 28.7 mm) was hanging from a horizontal twig by its feet and was grasping frog B (UVC 12730; SVL 27.9 mm) with its hands at the base of B's arms. B was grasping A in the same way, but was hanging with its feet free. The two frogs were facing the same direction.

B began swinging its body and grasped a leaf with its foot. After several minutes, it swung its body up and wrapped its feet and legs around A's body. At this point the two frogs were oriented venter-to-venter, hanging head down (fig. 1).

A then began to emit a call sporadically, consisting of a single, short, soft squeak lasting 0.14–0.15 s (fig. 2A). The frequencies of this call fall between 4000 and 7100 Hz and are strongly modulated. The emphasized frequency begins at around 4600 Hz and climbs to near 5000 Hz, at which point it jumps to about 7100 Hz and then rather abruptly falls to terminate near 6000 Hz. The first part of the call is notably pulsatile (although the number and pattern of pulses is variable), while the second, higher, part is only weakly so. When A called, the vocal sac expanded, which, in turn, pushed B from A, thereby loosening B's grip. After several minutes of this behavior, at 22.42 h, B fell to a leaf below, at which time it was collected. A climbed up onto the twig from which it had been hanging, and it was also collected. The observed combat lasted 37 min.

Both frogs were visibly wounded, presumably in combat. B, the "loser" of the encounter, had a red, swollen hematoma just dorsal and slightly anterior to the insertion of the right arm; the location of the injury corresponds to the position of A's humeral spine during combat. While A did not show any wounds or marks directly attributable to B's humeral spine, the skin

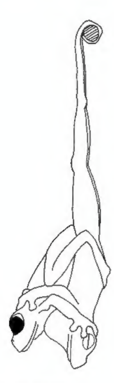


Fig. 1. – Illustration of combat in *Centrolene buckleyi*

on the dorsal surface of the outer edge of the right hand and fingers III and IV was torn. The right hand was observed not to be used when climbing in the plastic collecting bag. Both specimens were sluggish once collected.

DISCUSSION

The above description conforms well with descriptions of combat in *Cochranella griffithsi* Goin, 1961 (DUELLMAN & SAVITZKY, 1976) and *C. ignota* Lynch, 1990 (RESTREPO-TORO, 1996), both of which lack the humeral spine in males (although *C. griffithsi* males exhibit "a large bladelike ventral crest on the humerus"; LYNCH & RUIZ-CARRANZA, 1997: 529, fig. 3). Similar fighting has also been observed in *Centrolene prosoblepon* (Boettger, 1892) by JACOBSON (1985) and *C. acanthidiocephalum* (Ruiz-Carranza & Lynch, 1989) by Pedro M. RUIZ-CARRANZA (personal communication), two species which exhibit a humeral spine in males.

There are two differences between previous observations and ours. First, previous reports have not mentioned any evidence of physical damage inflicted by the humeral spine (although JACOBSON observed seven combat encounters). It is common to find scars on the head and body of males of *Centrolene geckoideum* Jiménez de la Espada, 1872 (personal

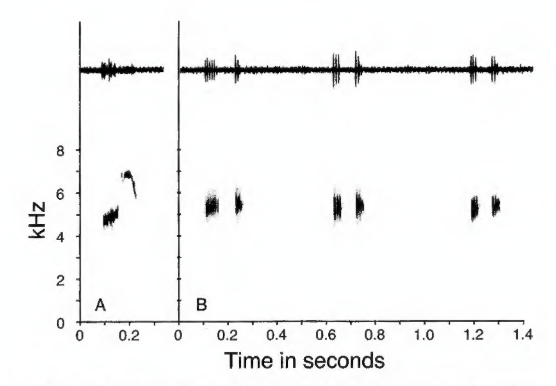


Fig. 2. – Audiospectrograms (graphed with wide-band 300-Hz filter) and waveforms of *Centrolene buckleyi* vocalizations, both recorded at 15.6°C air temperature, produced from tape copy on AMNH herpetology reel 276. (A) Combat call of UVC 12729. (B) Advertisement call of UVC 12589; *C. buckleyi* was observed to call sporadically, i.e., couplets are not usually emitted in series.

observation), presumably inflicted in combat by the extremely large and sharp spines of this species (as opposed to the blunt spine of *C. buckleyi*; see Ruiz-Carranza & Lynch, 1991, and Rueda-Almonacid, 1994, for spine shape and size in *C. buckleyi* and *C. geckoideum*, respectively), but to date combat has not been observed to confirm this suspicion. Our evidence of physical damage inflicted by the humeral spine is circumstantial (i.e., we did not examine the individual immediately prior to combat, so we cannot confirm the origin of the hematoma) but is more convincing than any previously reported.

The second difference is the use of a call during combat. Our interpretation of this event as non-accidental is based on the fact that we observed frog A to produce over 25 such calls (including 14 in recordings TG 9604 and 9605), all with the same effect. Inasmuch as the call – or, more precisely, the inflation of the vocal sac – appeared to be used to physically loosen the opponent's grip during combat, we suspect that the acoustic qualities of the call are unimportant. Despite this conjecture, the call varies remarkably little; all of the calls recorded exhibit essentially the same amplitude and frequency modulation as that shown in fig. 2A, i.e., it is not simply a random emission of sound made while inflating the vocal sac. As seen in fig. 2, this call differs markedly from the advertisement call (fig. 2B), which is a high-pitched,

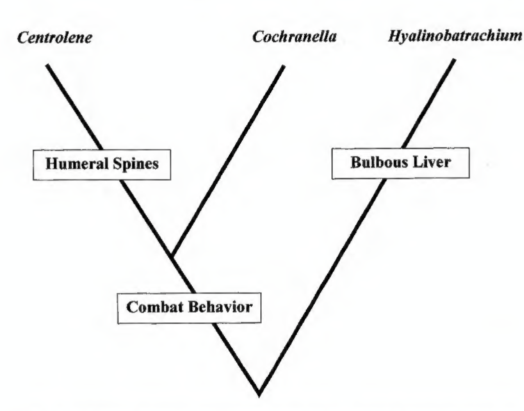


Fig. 3. – Conjectured phylogeny of centrolenid genera based exclusively on unique synapomorphies, i.e., character states that do not occur in any other anuran.

pulsed croak of 0.12–0.15 s duration consisting of two notes; the first note contains between three and six well defined pulses, while the second note contains three. The two notes are separated by 0.05–0.06 s intervals. The emphasized frequency lies at approximately 5200 Hz. The aggressive call also differs acoustically from the short, sporadic bursts of random noise that compose the encounter call of *Centrolene buckleyi* (fide John D. LYNCH, personal communication). Although B is an adult with vocal slits, it was not observed to call during the event.

The physical combat of frogs of the genus *Hyalinobatrachium* differs from that of *Centrolene* and *Cochranella*. MCDIARMID & ADLER (1974) described the combat behavior of *H. fleischmanni* (Boettger, 1893) (as *Centrolenella viridissima* Taylor, 1942) and *H. valerioi* (Dunn, 1931), in which one of the males grasps the other in amplexus; their description of *H. fleischmanni* combat was corroborated by GREER & WELLS (1980) and JACOBSON (1985). Strict outgroup comparison (sensu LYNCH, 1997: 355, footnote 2) reveals that this is the primitive behavior, while combat in which males dangle by their feet grappling venter-to-venter is derived.

Although the data set is exceedingly small (data are available for only 6.1 % of the family), it is sufficient to allow us to make a number of predictions based on a cladistic interpretation of known character distribution and published phylogenetic hypotheses (primarily Ruiz-Carranza & Lynch, 1991). First, we predict that the derived combat will be found in all 33 species of *Centrolene* for which combat remains to be observed. Similarly, we predict that none of the 24 uncoded species of *Hyalinobatrachium* will exhibit this derived state (i.e., they will exhibit either the plesiomorphic state or some other, unknown type of combat). Convincing evidence of monophyly has not been put forth for the more than 50 species (or any sizeable portion thereof) placed in *Cochranella*. However, the expression of the derived type of combat in two small but seemingly quite distantly related monophyletic groups of *Cochranella* – viz., the *ocellata* group sensu stricto (i.e., sensu Lynch, 1990) and the *griffithsi* group (sensu Lynch & Ruiz-Carranza, 1997: 529; named by Ruiz-Carranza & Lynch, 1995: 3) – is suggestive of a widespread distribution of this state throughout *Cochranella*. Consequently, we postulate (fig. 3) that the derived combat behavior constitutes a synapomorphy for *Centrolene* + (at least some part of) *Cochranella*, and therefore resolves the polytomy reported by Ruiz-Carranza & Lynch (1991). Data on the use of an aggressive call in combat are too limited ($n = 1$ species) to be phylogenetically informative at this time.

Resumen

En nuestras observaciones del combate físico de *Centrolene buckleyi*, los machos se colgaron de los pies y pelearon vientre-a-vientre. Un macho emitió repetidamente un chillido débil y corto, y así inflaba la bolsa vocal y empujaba al otro combatiente en el proceso. Los sonogramas de este canto agresivo y el canto de advertencia demuestran estructuras marcadamente diferentes. Cuando las colectamos, ambas ranas estaban visiblemente heridas, presumiblemente durante el combate. De las pocas especies codificadas por el comportamiento de combate ($n = 7$), *Centrolene* y *Cochranella* exhiben el estado derivado de colgarse de los pies y pelear vientre-a-vientre, mientras que *Hyalinobatrachium* presentan el combate primitivo en el cual un macho agarra al otro en amplexus. Predecimos que se descubrirá el comportamiento derivado en todas las especies de *Centrolene* y una gran parte de *Cochranella* (y por ende representa una sinapomorfia para estos dos grupos), y que ninguna especie de *Hyalinobatrachium* presentará el estado apomórfico.

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Universidad del Valle and donations to the Kelowna Museum by L. BELL and H. D. GRANT. Fernando CASTRO's efforts to promote herpetological investigations at the Universidad del Valle were the primary stimulus for our work.

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Análisis trófico en dos poblaciones de *Scinax nasicus* (Anura, Hylidae) de Argentina

Paola M. PELTZER & Rafael C. LAJMANOVICH

Instituto Nacional de Limnología (INALI-CONICET),
José Maciá 1933, Santo Tomé (3016), Santa Fe, Argentina

A comparative study of diets and morphometric analyses were made in *Scinax nasicus* in two localities of Santa Fe province (Argentina). A discriminant analysis was carried out to determine the morphometric variation of *S. nasicus*. The quantitative composition of diet for each locality was studied through the quantification of the trophic spectrum, niche trophic diversity and amplitude, prey size, as well as the Index of Relative Importance. Trophic relationships were obtained using overlap matrices based on Pianka's index. The results showed that *S. nasicus* has a strategic behavior for capture food between specialist and non-specialist ("sit-and-wait").

INTRODUCCIÓN

Scinax nasicus es un hílido que se distribuye en Argentina en las provincias de Corrientes, Chaco, Formosa, Entre Ríos, Jujuy, Salta, Santa Fe, Santiago del Estero y Tucumán; además, se encuentra en el sur de Bolivia, centro de Brasil, Paraguay y Uruguay (CEI, 1980; GALLARDO, 1987; LANGONE, 1994). Es frecuente hallarla asociada a ecosistemas periurbanos, en tanques de agua, piletas, u otros lugares húmedos durante la estación seca. En los ambientes del litoral mesopotámico argentino su reproducción ocurre generalmente en cuerpos de agua temporarios desde octubre hasta abril, dependiendo del régimen pluviométrico. Pone huevos que se encuentran sujetos, en forma de racimos gelatinosos, a plantas acuáticas (GALLARDO, 1987).

La información sobre datos bioecológicos de *S. nasicus* es dispar. Una primera aproximación al conocimiento de su dieta, en hábitats naturales de la provincia de Corrientes (Argentina), fue realizada por DURÉ & KEHR (1997). ORDANO et al. (1999) estudiaron sus hábitos alimentarios en ambientes antrópicos de Santa Fe (Argentina). La dieta larval fue descripta por LAJMANOVICH (1997) en ecosistemas del río Paraná, provincia de Santa Fe.

La relación entre caracteres biométricos y determinadas características biológicas en anuros ha sido tratada por diversos autores, e.g. EMERSON (1976, 1986), SALTHE & CRUMP (1977), WILBUR et al. (1978) y GATZ (1981).

Considerando que la utilización del alimento en los anfibios tiene un importante rol en la dinámica poblacional y en las interrelaciones interespecíficas, y que es un factor relevante para la evolución y organización de sus comunidades (CRUMP, 1974; DUELLMAN, 1978; TOFT & DUELLMAN, 1979, JONES 1982), el presente trabajo tiene como objetivos analizar la dieta de *S nasicus* en dos localidades de la provincia de Santa Fe y comparar las características morfométricas de las ranas, que permitan establecer variaciones geográficas en la especie.

MATERIALES Y MÉTODOS

ÁREAS DE ESTUDIO

Se colectaron manualmente un total de 50 adultos de *Scinax nasicus* durante el verano de 1996 en dos localidades de la provincia de Santa Fe, distantes aproximadamente a 300 km La fijación de los ejemplares se realizó in situ con una solución fijadora de formol al 10 %, inyectándose fijador en la cavidad abdominal con el fin de detener los procesos digestivos, tomando en cuenta que el tiempo que transcurre desde que los animales son capturados hasta su preservación puede afectar los resultados de los análisis de dieta (CALDWELL, 1996)

Según la clasificación de las regiones batracológicas propuestas por CEI (1980), el sitio Las Gamas se encuentra en la región Chaqueña y el sitio Colastine en la Litoral Mesopotámica. En la colección Herpetológica del Museo Provincial de Ciencias Naturales "Florentino Ameghino" de la ciudad de Santa Fe (Argentina) se conservan el total de ejemplares utilizados en este estudio (ap. 1)

Sitio Las Gamas, Dpto. Vera, Santa Fe (29°27'S, 60°23'O)

Fitogeográficamente se ubica en el Distrito Chaqueño Oriental (CABRERA, 1976). Se caracteriza por presentar bosques semixerófilos de *Schinopsis balansae* alternando con pastizales, esteros y bañados. Climáticamente, corresponde a una región tropical con estación seca, temperatura media anual de 20°C, precipitaciones anuales medias entre 950 y 1000 mm, y abundantes lluvias en verano (aproximadamente 300 mm)

Sitio Colastiné, Dpto. La Capital, Santa Fe (30°40'S, 60°30'O)

El área se sitúa en la zona sur de la llanura aluvial del río Paraná. El ambiente se caracteriza por presentar numerosos cursos de agua que forman una extensa red de drenaje con gran número de islas y cuerpos lénticos de distinta importancia, tales como lagunas, bañados y pantanos. Fitogeográficamente pertenece al Dominio Amazónico, Provincia Paranaense, Distrito de Selvas Mixtas (CABRERA, 1976), destacándose especies vegetales como *Salix humboldtiana*, *Acacia caven*, *Tessaria integrifolia*, *Croton urucurana* y *Sapium haematospermum*, entre otras. Climáticamente, el área corresponde a una zona subhúmeda-húmeda mesotermal con temperaturas medias anuales de 18°C y precipitaciones anuales medias de 1000 mm.

Metodología de laboratorio y análisis estadístico

En los ejemplares de ambos sitios se midieron longitudes de: hocico-cloaca (HC); ancho cabeza (AC); distancia interocular (DIO); borde anterior del ojo a la narina (BAON); mano, desde el tubérculo metacarpal externo al dedo más largo (LM); fémur (LF); tibia (LT); pie, desde el tuberculo metatarsal al dedo más largo (LP). Las medidas se tomaron con un calibre milimétrico de precisión 0,01 mm. A partir de estas medidas originales se determinaron sus proporciones con respecto a la longitud hocico-cloaca. En el análisis morfométrico, las mediciones se transformaron a su logaritmo natural con el fin de asegurar su distribucion normal y reducir la dispersión de los datos (Sokal & Rohlf, 1979).

La diferenciación de las poblaciones se realizó a través de un análisis discriminante entre los 4 grupos (ejemplares colectados en Las Gamas y en Colastiné, hembras y machos).

Los cálculos estadísticos se realizaron con el programa STATGRAPHICS® *Plus* For Windows (Anónimo, 1994).

Con el fin de analizar la dieta, los estómagos fueron disecados y estudiados individualmente. Para la determinación y cuantificación de los ítems alimentarios se consideraron como individuos aquellas estructuras o piezas claves para la identificación (cabezas, elitros etc.).

Para calcular la diversidad trófica de los contenidos estomacales se usó el método propuesto por Pielou (1966):

$$H = (1/N) \times (\log_2 N! - \Sigma \log_2 N_i!),$$

donde N es el número total de organismos hallados en el estómago de cada individuo y N_i es el número total de organismos de la especie i en cada estómago.

Se calculó la diversidad media (H) y la diversidad trófica acumulada (h_k) que se utiliza para determinar la muestra mínima en estudios herpetológicos (Hurtubia, 1973), según la siguiente fórmula:

$$h_k = (N_k H_k - N_{k-1} H_{k-1}) / (N_k - N_{k-1}),$$

donde H_k y H_{k-1} son las diversidades tróficas acumuladas en k y k-1 estómagos, y N_k y N_{k-1} son el número total de individuos de todas las especies presa en k y k-1 estómagos.

La amplitud trófica del nicho se obtuvo mediante el índice de Levins (1968):

$$Nb = (\Sigma P_{ij}^2)^{-1},$$

donde P_{ij} es la probabilidad de la proporción del ítem i en la muestra j.

Para establecer la contribución de cada categoría de alimento a la dieta, se aplico un índice de importancia relativa según Pinkas et al. (1971):

$$IRI = \% FO (\% N + \% V),$$

donde % FO es la frecuencia de ocurrencia de las categorías de alimentos, % N es el porcentaje numérico y % V el porcentaje volumétrico, calculado por desplazamiento de agua con una precisión de 0,01 ml.

La comparación de dieta de ambas poblaciones se elaboró en base al índice de solapamiento de PIANKA (1973):

$$S_{ij} = \Sigma P_{ij} P_{ik} / (\Sigma P_{ij}2 \Sigma P_{ik}2)^{½},$$

siendo P_{ij} y P_{ik} las proporciones en que los individuos j y k utilizan las diferentes clases que se reconocen en el recurso i. Este índice presenta valores que varían entre 0 y 1 en sentido creciente de coincidencia en la utilización de recursos.

RESULTADOS

SITIO LAS GAMAS

Del total de 25 individuos colectados, 10 fueron hembras y 15 machos. La comparación entre la proporción de sexos no fue significativa: Ji^2 (con corrección de Yates) = 0,7; $P > 0{,}05$. Los registros morfométricos del total de ejemplares colectados se presentan en la tab. 1.

El espectro trófico, basado en la identificación de 56 presas, resultó integrado por 22 taxa animales, restos de insectos no identificados y restos vegetales (tab. 2). La contribución de cada categoría de alimento a la dieta fue obtenida por la aplicación del índice de importancia relativa (IRI) (tab. 3, fig. 1), que presentó mayores valores en himenópteros y coleópteros. Las presas con mayor porcentaje de presencia fueron himenópteros (*Brachymirmex* spp.) (40 %); le siguieron las larvas de dípteros (36 %). Numéricamente, los himenópteros, con hormigas de la familia Dorylidae, son los más importantes (17,8 %), seguidos por las larvas de dípteros (15,8 %). Dentro de la fracción vegetal, el 40 % de los estómagos analizados presentaron resto de tallos y hojas, que no fueron evaluados numéricamente.

La diversidad media (H) resultó 0,26 ($s = 0{,}41$). La diversidad trófica acumulada (h_k) fue de 3,26. Con la suma de las 25 muestras, la curva de diversidad trofica tiende a la estabilización (fig. 2). La amplitud del nicho (Nb) para el periodo estudiado presentó un valor de 11,5. La distribución de frecuencias del tamaño de presas (fig. 3) presenta una distribución homogénea en los intervalos considerados.

En el intestino medio y posterior de 10 hembras y 2 machos se encontraron un total de 12 parásitos pertenecientes al phylum Nematoda.

SITIO COLASTINÉ

De los 25 ejemplares colectados, 12 fueron hembras y 13 machos. La comparación entre la proporción de sexos no fue significativa: Ji^2 (con corrección de Yates) = 0,083, $P > 0{,}05$. Los registros morfométricos del total de especímenes colectados se detallan en la tab. 1.

El espectro trófico, resultado de la identificación de 53 presas, estuvo integrado por 17 taxa animales, restos de insectos no identificados y restos vegetales (tab. 2). La contribución de cada categoría de alimento a la dieta (IRI) (tab. 3, fig. 1) mostró mayores valores en

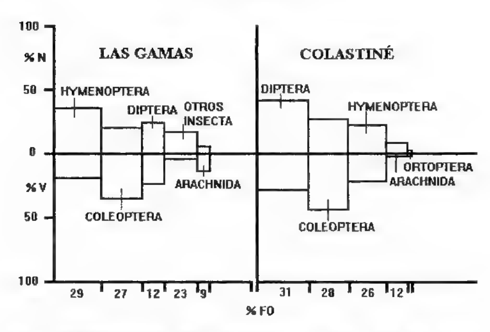


Fig. 1. – Representación gráfica del índice de importancia relativa (IRI) de los distintos componentes de la dieta de *Scinax nasicus* en la provincia de Santa Fe. % N, porcentaje numérico; % V, porcentaje volumétrico; % FO, porcentaje de ocurrencia.

Tabla 1. – Características morfométricas (en mm) evaluadas en *Scinax nasicus*. HC, longitud hocico-cloaca. Proporciones respecto de la longitud hocico cloaca: AC, ancho cabeza; DIO, distancia interocular; BAON, longitud desde el borde anterior del ojo y la narina; LM, longitud de la mano, desde tubérculo metacarpal externo al dedo más largo; LF, longitud fémur; LT, longitud tibia; LP, longitud del pie, desde el tubérculo metatarsal al dedo más largo. x, media; s_x, error estándar; s, desviación estándar; V, coeficiente de variación. Diferencia de medias: test t, $x_1 > x_2$. Significación: *, $P < 0{,}01$.

	Las Gamas				Colastiné				
	x_1	s_{x1}	s_1	V_1	x_2	s_{x2}	s_2	V_2	$x_1 > x_2$
HC	29,06	0,35	1,86	6,41	24,34	0,43	2,26	9,29	t = 8,29*
AC/HC	0,29	0,002	0,01	4,52	0,34	0,003	0,02	5,83	t = - 10,5*
DIO/HC	0,11	0,001	0,005	4,76	0,13	0,001	0,009	7,11	t = - 9,56*
BAON/HC	0,13	0,001	0,006	5,21	0,15	0,002	0,01	8,12	t = - 8,29*
LM/HC	0,27	0,003	0,01	5,73	0,3	0,004	0,02	8,07	t = - 4,79*
LF/HC	0,43	0,005	0,02	6,29	0,48	0,008	0,04	9,31	t = - 3,74*
LT/HC	0,5	0,006	0,03	6,26	0,55	0,006	0,03	6,48	t = - 5,34*
LP/HC	0,4	0,005	0,02	6,45	0,43	0,005	0,02	6,02	t = 4,33*

Tabla 2 - Dieta de *Scinax nasicus* en dos ambientes de la provincia de Santa Fe. *n*, número total de los 25 contenidos estomacales, %, porcentaje de la categoria en el total de las presas; *f*, frecuencia absoluta de la categoría en los estómagos, x, no evaluado numéricamente; (ni), no identificado

	Sitio Las Gamas			Sitio Colastiné		
	n	%	*f*	*n*	%	*f*
Categorias						
Insecta						
Coleoptera						
Carabidae	1	1,8	1	-	-	-
Coccinelidae	2	3,6	2	-	-	-
Curculionidae	1	1,8	1	-	-	-
Elateridae	4	7,14	4	1	1,89	1
Scarabaeidae	1	1,8	1	1	1,89	1
Hydrophilidae	1	1,8	1	3	5,66	2
Sylphidae	1	1,8	1	6	11,32	3
Dytiscoidea		-	-	1	1,89	1
(ni)		-	-	2	3,77	2
Hymenoptera						
Formicidae						
Acromyrmex spp.	2	3,6	3	6	11,32	5
Brachymirmex spp.	3	5,35	10	-	-	-
Dorylidae	10	17,85	3	2	3,77	1
Mirmicinae	3	5,35	3	-	-	
(ni)	3	3,35	2	4	7,54	3
Diptera						
Tabanidae (larvae)	3	5,35	3	-	-	
Chironomidae	-	-	-	5	9,43	2
Larvae (ni)	9	15,79	9	10	18,8	6
Pupae (ni)	-	-	-	3	5,66	1
(ni)	-	-	-	4	7,54	2
Orthoptera						
Tridactyloidea	1	1,8	4	-	-	-
Tettigonoidea	-	-	-	1	1,89	1
Homoptera						
(ni)	1	1,8	1	-	-	-
Hemiptera						
Corixidae	2	3,6	2	-	-	-
Lepidoptera						
Larvae (ni)	4	7,14	4	-	-	-
Apterygota						
Collembola	1	1,8	1	-	-	-
Arachnida						
Araneomorphae						
Caponiidae	1	1,8	1	2	3,77	2
Araneidae	1	1,8	1	-	-	-
Dictynidae		-		1	1,89	1
Acariformes	1	1,8	1	-	-	
Opilion (ni)	-	-	-	1	1,89	1
Restos de insectos (ni)	x	-	16	x	-	15
Restos vegetales	x	-	10	x		6
Total de presas	56			53		
(H)	0,26 (± 0,41)			0,22 (± 0,32)		
(Hk)	3,26			3,26		
(Nb)	11,52			10,6		
Tamaño promedio de presa por estomago	3,91 mm (± 0,61)			2,48 mm (± 0,22)		

Tabla 3. - Índice de importancia relativa (IRI) de los distintos componentes de la dieta de *Scinax nasicus*. % FO, porcentaje de frecuencia de ocurrencia; % N, porcentaje numérico; % V, porcentaje volumétrico.

Sitio Las Gamas				
	% FO	% N	% V	IRI = % FO (% N + % V)
Hymenoptera	29	37,05	19,5	1642
Coleoptera	27	19,64	36,5	1515
Diptera	12	21,42	24,5	554
Otros Insecta	23	16,07	4,8	488
Arachnida	9	5,35	14,7	147
Sitio Colastiné				
	% FO	% N	% V	IRI = % FO (% N + % V)
Diptera	31	41,5	28	2189
Coleoptera	28	26,41	44,5	1985
Hymenoptera	26	22,6	22	1159
Arachnida	12	7,5	3	120
Ortoptera	3	1,9	2,5	13,2

dípteros y coleópteros. Las presas con mayores porcentajes de presencia fueron las larvas de dípteros (24 %), y le siguen en orden de importancia las hormigas (*Acromyrmex* spp.) (20 %). Numericamente, las larvas de dípteros son las mas representadas (18,8 %). El 24 % de los estómagos contuvo restos de tallos y hojas.

La diversidad media (H) resultó 0,22 (s = 0,32). La diversidad trófica acumulada (h_k) fue de 3,26 y con la suma de las 25 muestras la curva de diversidad trófica tiende a la estabilización (fig. 2). La amplitud trófica del nicho (Nb) en el período de estudio fue de 10,6. La distribución de frecuencia del tamaño de presas (fig. 3) presentó una mayor concentración en el intervalo 1,5-3 mm.

En el intestino medio de un ejemplar macho se encontraron un total de 2 nemátodos.

Relación entre las poblaciones

Las comparaciones morfometricas realizadas entre los individuos provenientes de ambos sitios (tab. 1) arrojaron diferencias significativas en el 100 % de las medidas y relaciones evaluadas, las medias de Las Gamas son mayores que las de Colastiné.

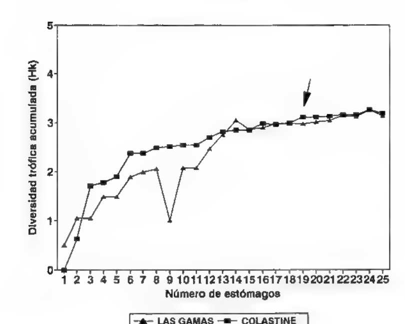


Fig 2 - Curva de diversidad trófica acumulada (H_k) versus número de estomagos analizados que determinan la muestra mínima para *Scinax nasicus* en las poblaciones Las Gamas y Colastiné La flecha sobre la curva indica aproximadamente el punto en donde se alcanza la estabilizacion.

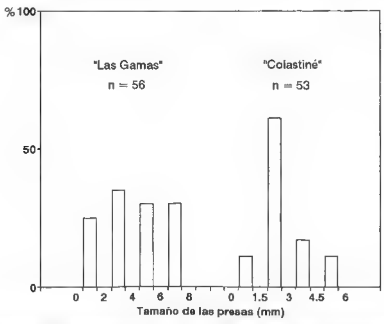


Fig 3 Distribucion de frecuencias de los tamaños de las presas consumidas por *Scinax nasicus* en Las Gamas y Colastine Sobre los histogramas figura el numero total de presas medidas (n)

El análisis discriminante entre los 4 grupos (ejemplares de Las Gamas y Colastiné, machos y hembras) explica en sus dos primeras funciones discriminantes (FD) el 96 % de la variación total. En la tab. 4 se representan los coeficientes de correlación canónicos estandarizados de cada variable. En la representación gráfica (fig. 4) quedan separados por las dos primeras FD los siguientes grupos: la mayoría de los individuos de la población de Las Gamas se sitúan en la parte positiva de las FD 1 y 2, y los de Colastiné en la negativa de la primera y positiva de la segunda. Con respecto al dimorfismo sexual, a pesar de notarse cierto alejamiento entre los grupos, no se aprecia una clara separación entre los sexos. La diferencia más destacable sería en relación a su longitud hocico-cloaca (hipótesis no comprobada).

El análisis de la dieta presentó una baja superposición en los ítems alimentarios (valor del índice de superposición de Pianka = 0,55).

Discusión

Hasta el momento no se han descrito subespecies de *Scinax nasicus*; sin embargo, *Scinax* es un género cuya taxonomía es compleja a causa de la importante variación que presentan sus especies (Faivovich, 1997). Este autor encontró diferencias osteológicas entre dos poblaciones de *Scinax fuscovarius* y entre dos poblaciones de *Scinax berthae*. Un análisis cladístico del genero *Scinax* en las especies argentinas fue realizado por Faivovich (1988), estudios que ampliarán el número de especies del género.

En las poblaciones investigadas, los resultados de los análisis morfometricos realizados no son evidencia suficiente para considerar que se trate de subespecies. Los efectos de la temperatura en el tamaño del cuerpo de los animales ectotermos son de difícil interpretación (Atkinson, 1996). La resolución a la paradoja de "porque los organismos usualmente son de mayor tamaño en ambientes más frios" está fundamentalmente relacionada con el aumento en el tamaño celular a bajas temperaturas (Van Voorhies, 1996; Atkinson & Sibly, 1997). Este fenómeno explica el incremento en el tamaño del cuerpo de los ectotermos a bajas temperaturas, independientemente de la ecologia específica de las especies. Estudios sobre la relación entre la aridez y el tamaño corporal, en anuros, no han encontrado relación entre las variables (Lee, 1993).

Los ejemplares provenientes de Las Gamas presentaron una importante proporción de hormigas y mayor diversidad de coleópteros en su dieta. En general se puede observar en los dos análisis un número de presas por estómago relativamente bajo en comparación con otras especies de anuros simpatricas estudiadas en la región (ver Lajmanovich, 1995, 1996). Los especimenes de Colastine, con una menor amplitud de nicho, predaron preferentemente sobre dipteros, coincidiendo con lo hallado por Duré & Kehr (1997) en la provincia de Corrientes, donde los órdenes mejor representados fueron los dípteros, himenópteros y coleópteros. Es menester aclarar que las características de los ambientes de la provincia de Corrientes concuerdan con los de Colastiné. En coincidencia con Duré & Kehr (1997), se considera que *S. nasicus* sigue una estrategia para capturar alimento intermedia entre forrajera y no especialista "sit-and-wait" (Huey & Pianka, 1981, Toft, 1981). Los especialistas son buscadores activos (por ejemplo, de hormigas), presentan glándulas venenosas y consumen muchas

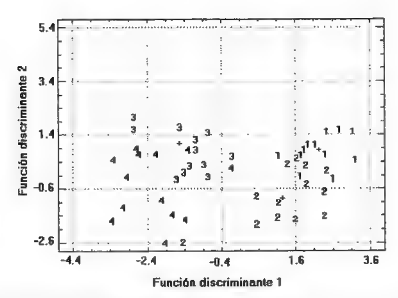


Fig. 4 - Resultado del análisis discriminante 1 y 2, machos y hembras de Las Gamas, 3 y 4, machos y hembras de Colastiné

Tabla 4. - Resultados del análisis discriminante. Ordenación de las variables según los dos primeros coeficientes de correlación canónicos (C.C.). HC, longitud hocico-cloaca. Proporciones respecto de la longitud hocico cloaca· AC, ancho cabeza; DIO, distancia interocular; BAON, longitud desde el borde anterior del ojo y la narina; LM, longitud de la mano, desde tubérculo metacarpal externo al dedo más largo; LF, longitud fémur; LT, longitud tibia; LP, longitud del pie, desde el tubérculo metatarsal al dedo más largo.

	C.C 1	C.C 2
HC	0,58	0,74
AC/HC	- 0,79	- 0,01
DIO/HC	-0,94	0,58
BAON/HC	0,33	-0,41
LM/HC	0,61	0,91
LF/HC	0,35	0,43
LT/HC	- 0,49	- 0,23
LP/HC	0,57	0,15

pequeñas presas por día; en contraposición, los no especialistas son depredadores inmóviles que esperan el paso de presas ocasionales (Toft, 1981). Los valores de amplitud trófica calculados se asemejan al hallado en otra especie generalista de la región (*Leptodactylus ocellatus*) (Lajmanovich, 1996).

Resumen

Se realizó un estudio comparativo de la dieta y de la morfometría de *Scinax nasicus* en dos localidades de la provincia de Santa Fe (Argentina). Mediante un análisis discriminante se establecieron variaciones morfométricas en la especie. Además, se cuantificó el espectro trófico, se calcularon la diversidad y la amplitud trófica del nicho, el tamaño de presa y el índice de importancia relativa. La comparación de las dietas de ambas poblaciones se obtuvo en base al índice de Pianka. Los resultados obtenidos muestran a *S. nasicus* con una estrategia para capturar alimento intermedia entre forrajera (especialista) y no especialista "sit-and-wait".

Agradecimientos

A Julián Faivovich por la colaboración en la colecta de los ejemplares de Vera y a Adolfo Beltzer por la lectura crítica del manuscrito. A Santiago Ron y muy especialmente a Analía Pugener por sus sugerencias y aportes bibliográficos.

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APÉNDICE I
MATERIAL ESTUDIADO

MFA-ZV-H: Colección Museo Florentino Ameghino, Zoologia Vertebrados, Herpetología, Santa Fe, Argentina.

Scinax nasicus

Sitio Las Gamas, Dpto. Vera, Santa Fe (29°27'S, 60°23'O): MFA-ZV-H 507 (lote de 25 ejemplares)

Sitio Colastiné, Dpto. La Capital, Santa Fe (30°40'S, 60°30'O). MFA-ZV-H 508 (lote de 25 ejemplares)

The life-history traits of *Eurycea guttolineata* (Caudata, Plethodontidae), with implications for life-history evolution

Jeremy L. MARSHALL [1-2]

Department of Biology, University of Southwestern Louisiana,
P.O. Box 42451, Lafayette, Louisiana, 70504-2451, USA
E-mail: jmarshal@usl.edu

Evaluating life-history traits allows for the assessment of local adaptation and its correlated fitness consequences. The goal of this study was to describe the life-history traits of a spring-dwelling population of *Eurycea guttolineata* to gain a better understanding of life-history evolution in the Plethodontidae. Size at first reproduction, ≥ 50.00 mm SVL, was similar between males and females and was attained at 22-24 months of age. However, a larger variance in size of sexually mature females (about twice male variance) may suggest that some females do not become sexually mature until 34-36 months of age. The data suggest a period of sexual activity from late summer to early winter (July-December), ovipositing occurring in early winter (November-December), and egg hatching probably occurring in January or February. During ontogeny, growth rates were high during the first (2.48 mm SVL/mon) and second (1.70 mm SVL/mon) years, but decreased (0.11 mm SVL/mon) once sexual maturity was reached. I found that metamorphosis occurred typically in June, at a size of 23.08 mm SVL, at 5-6 months of age. A coefficient of variation analysis revealed that age at metamorphosis was significantly more variable than size. This, in conjunction with the fast larval growth rates and short larval period of this species, is consistent with a hypothesis based on larval adaptation to warm, stable aquatic environments in which an optimal size at metamorphosis is reached at an early age. This analysis does not support the hypothesis that larvae of this species are adapted to uncertain environments.

INTRODUCTION

Evaluating life-history traits across the geographic distribution of a species is critical to interpreting the influence of local environments on life-history variation (STEARNS, 1992; TILLEY & BERNARDO, 1993). Such variation in life-history traits may reflect phylogeny

1. Work completed at Department of Biology, University of Mississippi, University, Mississippi 38677, USA
2. This paper is dedicated to the memory of Nick PIETROPAOLO.

(Harvey & Pagel, 1991) or may represent adaptation to local environments (Levins, 1968, Stearns, 1992) Life-history studies that address phylogenetic history and local adaptations are now being conducted at the level of genus and species with comparative methods (Bauwens & Diaz-Uriarte, 1997; Irschick & Losos, 1998). However, a fundamental criterion for evaluating the evolution of life-history traits, with comparative methods, is that such traits are known for each of the taxa or populations under consideration (Harvey & Pagel, 1991).

Life-history traits of the genus *Eurycea* (Caudata, Plethodontidae) have been documented from across the eastern United States (*E. longicauda*, Anderson & Martino, 1966; *E. multiplicata*, Ireland, 1974; *E. quadridigitata*, Semlitsch & McMillan, 1980, *E. junaluska*, Sever, 1983; *E. wilderae*, Bruce, 1988; *E. cirrigera*, Marshall, 1997; *E. lucifuga*, Carlyle et al., 1998). Considerable intraspecific variation in life-history traits has been observed, especially within those species that inhabit a wide variety of habitats (Tilley & Bernardo, 1993; Voss, 1993, Marshall, 1996, 1997, Carlyle et al., 1998). Habitat differences are the impetus for local adaptation and may lead to the evolution of novel life-history characteristics (e.g., Bahert, 1996; Marshall, 1996). Therefore, the assessment of life-history traits among closely related species or populations within different habitats and regions should illuminate potential sources of life-history variation (Berven, 1982; Tilley & Bernardo, 1993).

The three-lined salamander, *Eurycea guttolineata* Holbrook, 1838, (formerly *E. longicauda guttolineata*) was raised to specific status by Carlin (1997). This species has a bi-phasic life cycle (Conant & Collins, 1991; Duellman & Trueb, 1994) and inhabits a wide variety of seepage, spring, river swamp, and creek systems in the eastern United States (Conant & Collins, 1991) The life-history traits of *E. guttolineata* and *E. longicauda* have been studied in a variety of geographic locations. The traits of larvae and just metamorphosed specimens of *E. guttolineata* from a spring-fed marsh in North Carolina were described by Bruce (1982), while some of the developmental and reproductive characteristics from a flood plain population in Florida were described by Gordon (1953). A detailed life-history study of *E. longicauda* inhabiting temporary ponds in New Jersey was conducted by Anderson & Martino (1966) Ireland (1974) described the life-history traits of *E. l. melanopleura* from a spring-fed pond in Arkansas.

The previously studied populations of *E. guttolineata* were located largely in ephemeral habitats. I examined a population of *E. guttolineata* that inhabits an annually invariant, stenothermic spring ecosystem in the coastal plain of northern Mississippi, USA. The goals of my study were to describe the life-history traits of this spring-dwelling population of *E. guttolineata*, compare the findings to the results from other populations, and evaluate the influence of intra- and interspecific variation on the evolution of life-history characteristics among members of the *Eurycea longicauda* complex.

Materials and methods

The study site was Poplar Cove, an approximately 50 m^2 spring, located at The University of Mississippi Biological Field Station in the North-Central Hills physiographic province of

Lafayette County, Mississippi, USA. Year round, the spring was stenothermic ($x \pm s$ = 16.9 ± 1.8°C), with dissolved oxygen levels ranging from 0.7 to 9.4 ppm ($x \pm s$ = 7.28 ± 1.79 ppm). Water depths ranged from 0.005 to 0.100 m ($x \pm s$ = 0.033 ± 0.02 m). The area surrounding Poplar Cove Spring (PCS) was a mixed pine-hardwood forest comprised of short-leaf pine (*Pinus echinata*), eastern red cedar (*Juniperus virginiana*), blackjack oak (*Quercus marilandica*), southern red oak (*Q. falcata*), water oak (*Q. nigra*), white oak (*Q. alba*) and sycamore (*Platanus occidentalis*). The immediate area of the spring had a canopy dominated by tulip poplar (*Liriodendron tulipifera*), an understory of American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), giant cane (*Arundinaria gigantea*), American holly (*Ilex opaca*), and the herbaceous plants netted chain-fern (*Woodwardia areolata*) and lizard's tail (*Saururus cernuus*). At this site, *E. guttolineata* co-occurred with several other caudates, including the southern two-lined salamander (*E. cirrigera*), the red-spotted newt (*Notophthalmus viridescens*), the Mississippi slimy salamander (*Plethodon mississippi*), the red salamander (*Pseudotriton ruber*) and the lesser siren (*Siren intermedia*), although the latter two species were rarely seen.

I installed a 35 m long drift fence constructed of 0.61 m wide aluminum flashing. The fence began at the point of emergence of the spring and lay approximately 4 m from the spring's margin. The drift fence bordered approximately three-fourths of the total margin but did not impede water flow. The bottom of the fence was buried to a depth of 0.10 m. The fence was supported at 3.0 m intervals with two 0.50 m lengths of 0.02 m diameter PVC piping fastened by plastic electrical ties. I placed pitfall traps adjacent to, and on each side of, the fence at approximately 3.0 m intervals, with single-ended funnel traps placed at the ends because of soil saturation in those locations. Coverboards (0.62 × 0.19 × 0.025 m wooden planks) were then placed in between pitfall traps at certain locations along the fence. The pitfall traps were 944 ml plastic buckets (0.115 m in diameter) with a 0.025 m internal lip to help prevent escape (sensu DODD & SCOTT, 1994).

Daily surveys of the drift fence were conducted from April 1995 to December 1996. As this research was part of a larger life-history study on caudates, *E. guttolineata* measurements were taken rarely during 1995. However, more thorough measurements were taken during 1996. In addition to the daily surveying along the drift fence at PCS, samples of aquatic and terrestrial salamanders were collected in May, July, August and November 1996. The aquatic samples were conducted with the aid of a dip net. The terrestrial samples were taken with the aid of a potato rake for searching through ground litter. The time spent surveying the aquatic (180 min) and terrestrial (60 min) habitats was relative to their total area (i.e., the aquatic and terrestrial habitats were 50 and 17 m^2, respectively). This method was used to reduce the bias of sampling any particular area unequally. All survey data were used for determining activity, sizes, ages and months of metamorphosis and sexual activity.

I determined mean body sizes (to the nearest 0.01 mm SVL) of larvae, juveniles and adults on a monthly basis. Reproductive status of adults was determined by the presence of yolked oocytes in females (seen through the venter) and secondary sexual characteristics, such as nasal cirri and mental glands, in males (ARNOLD et al., 1993; DUELLMAN & TRUEB, 1994). I compared SVL of just metamorphosed and adult individuals within and between years with the Mann-Whitney *U* and Kruskal-Wallis *H* tests (ZAR, 1984). Size classes of individuals were established from the monthly data.

Based on the size class data from the monthly samples at PCS, ages were estimated and then assigned for each individual This was accomplished by utilizing the three size classes of individuals (see fig. 1a and 2b, May-August) and assigning ages between 0 and 11 months for the first, 12 and 23 months for the second, and 24 and 35 months for the third size class, respectively. Larval hatching was assumed to occur in January based on the presence of a few newly hatched larvae at PCS. Larvae found in January were assigned an age of zero month. Although there may be some error in the estimates of older age classes (i.e., ≥ 31 months of age), this technique provides an adequate method for assigning respective ages of larval, juvenile and subadult salamanders with non-overlapping size classes (BRUCE, 1988; STEARNS, 1992).

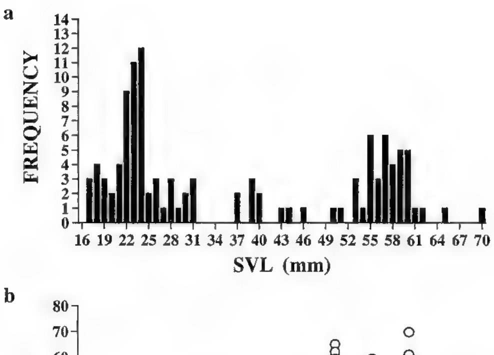
I estimated growth rates by regressing month of capture versus size (SVL) for each size class of individuals (ZAR, 1984). A general model of growth over the first 35 months of life was estimated by regressing estimated age versus SVL. This approach allowed for the general assessment of larval period, juvenile period, age and size at metamorphosis, and age and size at sexual maturity. I then compared these life-history characteristics to those of other populations of *E. guttolineata* and *E. longicauda*.

I utilized a Haldane coefficient of variation analysis for samples, i.e., $V_H = (1 + 1/4n)(s/x)$, to determine differences in variation of age and size at metamorphosis among populations of the *E. longicauda* complex. This analysis corrects for the bias of small sample size and the effects of sampling (HALDANE, 1955; SOKAL & BRAUMANN, 1980; DELAUGERRE & DUBOIS, 1985). A *F* ratio test was used to determine statistical significance between coefficient of variation values (LEWONTIN, 1966) Assumptions of normality were met for all analyses. Finally, the relationships between larval growth rate, size at metamorphosis, and age at metamorphosis, were assessed intra- and interspecifically among populations of the *E. longicauda* complex. Growth rates were estimated using BEACHY's (1995*a*) equations. Relationships were evaluated using regression and correlation coefficient analyses (ZAR, 1984). Statistical significance was set at $\alpha = 0.05$.

RESULTS

The population structure of the 1995 (n = 48) and 1996 (n = 61) samples of *E. guttolineata* from PCS revealed two juvenile age classes prior to the first adult age class (fig. 1b). Mean sizes for each age class and month are presented in tab. 1. A few newly hatched larvae were collected in January 1995, although not measured Larvae were present at PCS through May (tab. 1). I found that metamorphosis occurred in June and July (tab. 1). This corresponds to an age at metamorphosis of 5-6 months, assuming hatching occurred in January. After metamorphosis, the juvenile period lasted 17-19 months.

The data on sexually mature individuals (tab. 1) indicated a late summer to late autumn (July-October) period of sexual activity, with the smallest females becoming sexually active during the latter part of the season (see tab. 1, October and December) The smallest female at sexual maturity was 50 5 mm SVL I estimated the age of this individual to be 23 months. Therefore, age at first reproduction is reached at the end of the second year of life at 22-



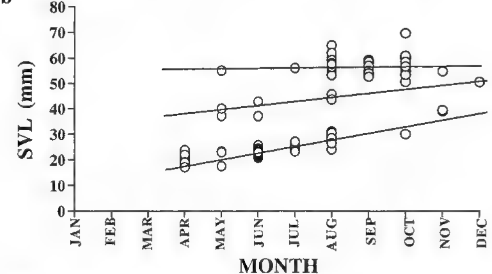


Fig. 1 (*a*) The frequency distribution of snout-vent lengths (SVL) of *Eurycea guttolineata* from the 1995 and 1996 pooled data from Poplar Cove Spring in Lafayette Co., Mississippi, USA. The three designated size classes are 17-31 mm, 37-46 mm and 50-70 mm SVL. (b) The pooled population structure of the 1995 ($n = 48$) and 1996 ($n = 61$) monthly samples of *E. guttolineata*. Growth rates are based on the regression slope for first and second year juveniles, as well as the adult estimate (solid lines). The regression analyses for each growth period were as follows: first year growth, slope = 2.48, $r^2 = 0.84$, $df = 65$, $F = 326.79$, $P = 0.0001$; second year growth, slope = 1.70, $r^2 = 0.82$, $df = 7$, $F = 27.41$, $P = 0.0019$; adult estimate, slope = 0.11, $r^2 = 0.001$, $df = 37$, $F = 0.04$, $P = 0.8356$

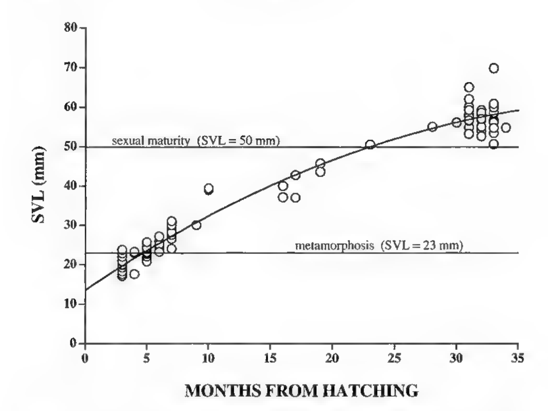


Fig. 2. – A general growth model for *E. guttolineata* (n = 109), from the pooled data of 1995 and 1996. This model incorporates timing of metamorphosis and sexual maturity. Individual salamanders were assigned an age based on their size and month of capture. These age classed data were then used to generate this growth model. A second order polynomial regression was used to generate this model. The model is size (SVL) = – 0.023 age^2 + 2.121 age + 13.506, r^2 = 0.971.

24 months of age. However, the majority (85 %) of sexually mature individuals were probably at least 30 months of age with a SVL ≥ 55.00 mm. The grand mean, standard deviation, range and coefficient of variation (V_H) for size (mm SVL) of sexually mature individuals are as follows: males, n = 23, $x \pm s$ = 56.99 ± 2.12, range 52.60-60.80, V_H = 3.79; females, n = 14, $x \pm s$ = 58.29 ± 5.35, range 50.50-69.80, V_H = 9.51. Females exhibited significantly more variation in size than males ($F_{14,23}$ = 5.95, P < 0.001). Egg-laying probably occurred from November to January based on the disappearance of females during late autumn and the presence of new hatchlings in January (although I observed no egg masses).

There was no difference in size at metamorphosis between samples from June 1995 and 1996 (1995, n = 25, $x \pm s$ = 22.98 ± 1.09, range 20.76-25.32, 1996, n = 3, $x \pm s$ = 23.94 ± 1.93, range 21.89-25.71; U = 23, P = 0.2815). There was also no difference in grand mean female SVL between 1995 and 1996 samples (1995, see tab. 1; 1996, n = 9, $x \pm s$ = 56.65 ± 5.94, range 50.5-69.8, U = 8, P = 0.0532). Sexually active males in the 1996 sample exhibited no monthly differences in mean SVL (H = 2.24, df = 2, P = 0.327). The lack of significant differences

Table 1. - SVL (in mm) for each age class of *Eurycea guttolineata* for each month of 1996. For each sample, the table gives $x \pm s$, followed by n in parenthesis. Data are from Poplar Cove Spring, Lafayette County, Mississippi, USA. *Italics*, data from the 1995 sample only. **Bold**, pooled data from 1995 and 1996.

Age class	April	May	June	July	August	Septem.	October	Novem.	Decem.
Larvae	*19.51 ± 2.08 (16)*	*23.09 ± 0.21 (2)*	-	-	-	-	-	-	-
1st year juveniles	-	-	**23.08 ± 1.19 (28)**	25.06 ± 1.53 (5)	28.62 ± 2.21 (10)	-	30.03 ± 0.00 (1)	39.20 ± 0.28 (2)	-
2nd year juveniles	-	39.04 ± 1.66 (3)	39.93 ± 4.05 (2)	-	44.67 ± 1.50 (2)	-	-	-	-
Adult males	-	-	-	-	56.12 ± 1.79 (6)	56.97 ± 2.09 (9)	58.05 ± 2.26 (7)	54.74 ± 0.00 (1)	-
Adult females	-	55.00 ± 0.00 (1)	-	56.07 ± 0.00 (1)	*61.25 ± 2.33 (5)*	54.00 ± 0.00 (1)	58.21 ± 6.67 (6)	-	50.50 ± 0.00 (1)

between the 1995 and 1996 samples justified pooling these data for use in growth analyses (SOKAL & ROHLF, 1995).

The solid lines in fig. 1b represent growth rates for first and second year juveniles, and adults. The first year rate of growth (2.48 mm SVL/mon) incorporates both larval and juvenile growth (see fig. 1b and tab. 1 for data). The y-intercept of the first year growth equation (11.21 mm) closely estimates published SVL data for hatchlings of *E. guttolineata* from other populations (GORDON, 1953; ANDERSON & MARTINO, 1966, BRUCE, 1970, 1982). Second year growth (1.70 mm SVL/mon) decreased from the first year, while estimated adult growth was minimal (0.11 mm SVL/mon). The combined rate of growth for juveniles, i.e., from metamorphosis to sexual maturity, was 1.49 mm SVL/mon. I generated a predictive growth model for *E. guttolineata*, based on estimates of age and measures of size, that incorporates timing of metamorphosis and maturity (fig. 2).

I summarized life-history data on size, age and timing of metamorphosis from 10 populations within the *E. longicauda* complex (tab. 2). Age at metamorphosis data from each population incorporated potential variation from egg-laying dates. Using these data, I calculated the Haldane coefficient of variation for both age and size at metamorphosis. The mean data for size (tab. 2) were not different from the raw data with respect to the mean or variance ($t = 0.95$, $df = 127$, $P = 0.344$, mean data, $n = 10$, $V_H$size = 9.53; raw data, $n = 119$, $V_H$size = 8.54; $F_{10,119} = 1.293$, $P > 0.50$). I used data from the first year's metamorphosing

Table 2. - Data on metamorphosis for populations of the *Eurycea longicauda* complex. *E. g.*, *E. guttolineata*; *E. l. l.*, *E. l. longicauda*; *E. l. m.*, *E. l. melanopleura*. SM, size at metamorphosis (mm SVL). AM, age at metamorphosis (months). Rate, larval growth rate (mm/month). MM, month(s) when metamorphosis occurs. Perm, permanent habitat type. Ephl, ephemeral habitat type. *Italics*, standard deviation from a larval sample with same size range as just metamorphosed individuals. **Bold**, standard deviation estimated from mean and range. Sources: (1) this study; (2) Bruce, 1982 (Caney Fork); (3) Bruce, 1970: (3a) Cox Cove, (3b) Horse Cove; (4) Gordon, 1953, (5) Sinclair, 1951, (6) Anderson & Martino, 1966, (7) Franz & Harris, 1965; (8) Ireland, 1974; (9) Rudolph, 1978.

Species	State: county	*n*	SM: $x \pm s$ (range)	AM: *x* (range)	Rate	MM	Habitat	Source
E. g.	MS: Lafayette	28	23.08 ± 1.19 (20.71-25.71)	5.50 (5.0-6.0)	2.378	Jun-Jul	Perm	1
E. g.	NC: Jackson	9	25.70 ± 0.71 (25.00-27.00)	4.50 (4.0-5.0)	3.044	Jul-Aug	Perm	2
E. g.	NC: Jackson	5	24.40 ± 0.89 (23.00-25.00)	3.75 (3.5-4.0)	3.840	Jun	Ephl	3a
E. g.	NC: Macon	9	26.60 ± 2.96 (23.00-32.00)	4.50 (3.5-5.5)	3.689	Aug	?	3b
E. g.	FL: Jackson	1	21.00	6.50 (6.0-7.0)	1.692	Jun-Jul	Ephl	4
E. g.	TN: Haywood	2	23.25 ± 1.06 (22.50-24.00)	?	?	Jun	?	5
E. l. l.	NJ: Sussex	18	22.50 ± **1.15** (20.20-24.50)	3.50 (3.0-4.0)	3.742	Jun	Ephl	6
E. l. l.	MD: Garrett	15	19.50 (18.00-21.00)	?	?	Jul	Perm	7
E. l. m.	AR: Washington	24	25.50 ± *1.25* (23.00-28.00)	6.00 (5.0-7.0)	2.583	Jun-Jul	Perm	8
E. l. m.	OK: Delaware	25	24.28 ± 2.25 (19.00-29.00)	6.50 (4.0-9.0)	2.197	Jul-Oct	Perm	9
Grand means, ranges, totals		136	23.58 ± 2.19 (18.00-32.00)	5.09 (3.5-9.0)	2.720	Jun-Oct	-	-

populations only, as this was a more conservative measure of variation in age at metamorphosis. Including individuals that over-wintered, i.e., > 12 month larval period, increased the coefficient of variation for age more than size. I found that age at metamorphosis had a significantly greater coefficient of variation than size at metamorphosis within the complex ($V_H$age = 24.19, $V_H$size = 9.53, $F_{8,10}$ = 5.81, $P < 0.05$). Moreover, this finding was consistent when habitat type (i.e., populations occurring either in permanent or ephemeral habitats) was included in the analysis (permanent, $V_H$age = 16.13, $V_H$size = 5.24, $F_{4,4}$ = 10.09, $P < 0.05$; ephemeral, $V_H$age = 39.35, $V_H$size = 8.16, $F_{3,3}$ = 18.87, $P < 0.05$). In contrast, populations of *E. quadridigitata*, the dwarf salamander, which utilize ephemeral habitats, have significantly greater variation in size than age at metamorphosis ($V_H$age = 7.37, $V_H$size = 22.41, $F_{5,5}$ = 10.58, $P < 0.05$, data from: Bishop, 1947, Harrison, 1973; Semlitsch, 1980, Dundee & Rossman, 1989).

Finally, I analyzed the relationships between larval growth rate, size at metamorphosis, and age at metamorphosis within and among species in the complex (fig 3a-c). I found that among populations there was not a significant relationship between larval growth rates and size at metamorphosis ($r = 0.46$, $P > 0.20$; H_0: b = 0, $t = 1.28$, $P = 0.2489$) and age at metamorphosis and size at metamorphosis ($r = 0.23$, $P > 0.50$, H_0. b = 0, $t = 0.57$, $P = 0.5869$) However, there was a significant relationship between larval growth rate and age at metamorphosis among populations ($r = 0.94$, $P < 0.001$; H_0: b = 0, $t = 6.74$, $P = 0.0005$). When the data were analyzed within species, only data from populations of *E. guttolineata* provided sufficient sample sizes. Among populations of *E. guttolineata*, there was a significant correlation between larval growth rates and size at metamorphosis ($r = 0.85$, $P < 0.02$; H_0: b = 0, $t = 1.84$, $P = 0.0701$), larval growth rate and age at metamorphosis ($r = 0.96$, $P < 0.001$; H_0: b = 0, $t = 6.23$, $P = 0.0084$), and age at metamorphosis and size at metamorphosis ($r = 0.80$, $P < 0.05$; H_0: b = 0, $t = 2.33$, $P = 0.1018$). However, only the relationship between larval growth rate and age at metamorphosis was significantly different from the null hypothesis b = 0 (see above). The relationships between these traits for *E. l. longicauda* and *E. l. melanopleura* are shown in fig. 3a-c.

DISCUSSION

The life-history traits of this population of *E. guttolineata* were similar to other taxa and populations in the *E. longicauda* complex. The larval period of this population was comparable to North Carolina and Florida populations of *E. guttolineata* and a population of *E. l. melanopleura* in Arkansas, but longer than that of *E. l. longicauda* from New Jersey (tab. 2). Metamorphosis also appeared to take place at a similar time regardless of the population (tab. 2). This semi-consistent pattern of timing of metamorphosis may be a function of phylogenetic history among these closely related populations, i.e., a relatedness constraint. However, there was variation in age at metamorphosis among populations, which was significantly more variable than size at metamorphosis. Therefore, variation in age at metamorphosis could result from plasticity in growth rates, as a function of the habitat, to reach an optimal size at metamorphosis (WILBUR & COLLINS, 1973) and/or genetically based differences in age at metamorphosis among populations (BERVEN, 1982)

Previous studies suggest that the short larval period of members of the *E. longicauda* complex reflects an adaptation to uncertain/ephemeral aquatic environments (ANDERSON & MARTINO, 1966; BRUCE, 1982). To evaluate this hypothesis, some theoretical predictions should be considered. WILBUR & COLLINS (1973) stated that species that exploit certain/permanent environments should have a narrow range of sizes at metamorphosis (i.e., around an optimum) and a greater range in age at metamorphosis. This pattern should result in increased variation in age at metamorphosis (e.g., from a few months to a year). In contrast, those species which exploit uncertain/ephemeral habitats should exhibit the opposite trend (WILBUR & COLLINS, 1973) Moreover, if selection is favoring an optimal size at metamorphosis, then growth rates should only influence the time it takes to reach an optimal size. BRUCE (1982) elaborated on WILBUR & COLLINS's (1973) model by stating that in uncertain environments slower growing larvae should metamorphose at a smaller size, as opposed to delaying metamorphosis until the optimal size is reached These theoretical predictions

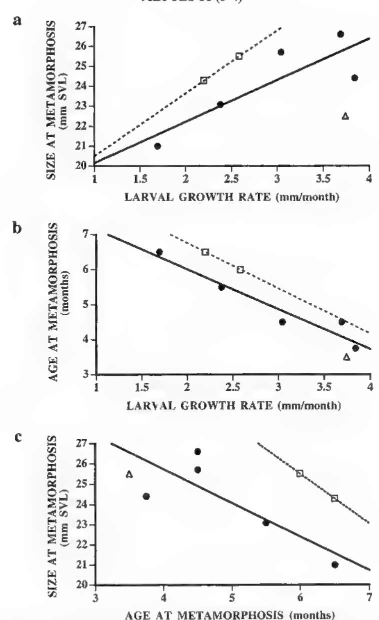


Fig 3 - Relationships between larval growth rate, age at metamorphosis, and size at metamorphosis for each member of the *E. longicauda* complex. Open squares and dashed lines, *E. l. melanopleura*, solid circles and lines, *E. guttolineata*, open triangles, *E. l. longicauda*. (a) Relationship between larval growth rate and size at metamorphosis for each species. *E. guttolineata*, size (SVL) = 2.075 rate + 18.078, r^2 0.718, *E. l. melanopleura*, size (SVL) = 3.161 rate + 17.336. (b) Relationship between larval growth rate and age at metamorphosis. *E. guttolineata*, age (months) 1.139 rate + 8.256, r^2 0.928, *E. l. melanopleura*, age (months) = 1.295 rate + 9.346. (c) Relationship between age and size at metamorphosis. *E. guttolineata*, size (SVL) = 1.664 age + 32.393, r^2 - 0.645, *E. l. melanopleura*, size (SVL) = −2.440 age + 40.140.

provide the basis for my evaluation of the hypothesis of an adaptation to uncertain environments for this complex.

The findings of this study, that age at metamorphosis is significantly more variable than size at metamorphosis, do not support the hypothesis of adaptation to uncertain environments. Instead, the data support the alternative prediction of WILBUR & COLLINS's (1973) model, which states that in stable environments individuals should remain in the aquatic environment until an optimal size at metamorphosis is reached. The significant relationship (i.e., correlation coefficient and b) between larval growth rate and age at metamorphosis, but not larval growth rate and size at metamorphosis (i.e., b = 0), supports the latter prediction. Both within and among species in this complex, the relationship between age at metamorphosis and size at metamorphosis was not significantly different from the null hypothesis b = 0. In addition, there is corroborating evidence that several populations within the *E. longicauda* complex have fast growing larvae that metamorphose within months of hatching and slow growing larvae that metamorphose more than 12 months after hatching (FRANZ, 1967, RUDOLF, 1978, BRUCE, 1982). Moreover, populations of *E. quadridigitata* that inhabit ephemeral habitats exhibited the opposite trend (i.e., significantly greater variation in size than age at metamorphosis). Therefore, populations of the *E. longicauda* complex meet the predictions of WILBUR & COLLINS's (1973) model and support, at least in part, the hypothesis of selection for an optimal size at metamorphosis.

Although the data do not support the hypothesis of an adaptation to uncertain environments, the hypothesis of selection for an optimal size at metamorphosis does not address directly why members of this complex have shorter larval periods and smaller sizes at metamorphosis relative to other semi-aquatic plethodontids. One evolutionary explanation is that larvae are adapted to stable, warmer aquatic environments with increased food regimes (e.g., food availability), resulting in increased growth rates and smaller sizes at metamorphosis (BEACHY, 1995*b*).

Several studies have shown that increases in temperature and food result in increased larval growth rates (WILDER, 1924; STEWART, 1956; BIZER, 1978; SEXTON & BIZER, 1978; BEACHY, 1995*b*). However, a conflict, over the influence that increased temperature has on size at metamorphosis, has arisen between alternative models of metamorphosis. SEXTON & BIZER (1978) stated that increases in temperature should result in shorter larval periods and smaller sizes at metamorphosis. However, JUTERBOCK (1990) stated that temperature influences on growth are not consistent among plethodontids (e.g., that sometimes decreases in temperature result in smaller sizes at metamorphosis). BEACHY (1995*b*) stated that the discrepancies could be accounted for by the complex relationship between increased temperatures and food regimes (i.e., that increases in temperature are accompanied by increases in food regimes). This complex temperature-food interaction can allow for increased larval growth rates, shorter larval periods, and a range of sizes at metamorphosis. This reconciles the question of how an optimal size at metamorphosis, facilitated by a stable environment, can be accompanied by a shorter larval period. A warmer, more stable aquatic environment would allow an optimal size at metamorphosis to be reached at an earlier age through an increased growth rate. Therefore, the data support the notion that habitat parameters (such as temperature and food) directly influence larval growth rates, which then influence the age at which an optimal size at metamorphosis is reached.

The majority of plethodontid life-history theory has centered on the genus *Desmognathus* (for a review, see Tilley & Bernardo, 1993). However, the dominant theory for the desmognathines, that increased adult body sizes are due to increased ages at maturation, does not hold for salamanders in the genus *Eurycea*. *Eurycea guttolineata* and its close relatives are at least 20 mm SVL larger (Bruce, 1982, Conant & Collins, 1991; this study) and become sexually mature sooner than or at the same age as other salamanders in the genus (i.e., *E. bislineata* complex). This suggests that age at maturity could not account for the differences in adult body size. Moreover, it appears that juvenile growth rate, juvenile period, and/or size at maturation, account for the differences in adult body size within this genus (Marshall, unpublished data). Although different taxa in the family Plethodontidae appear to be utilizing different strategies to attain larger body sizes, the influence of aquatic habitats on larval development may be consistent among genera (i.e., increases in temperature result in increase in larval growth rates). Moreover, this analysis provides evidence that intra- and interspecific variation in life-history traits is influenced by local environments, which play a critical role in shaping life-history evolution.

Resumen

La evaluación de características de la historia de vida nos permiten estimar la adaptación local y sus consecuencias correlacionadas de ajuste. El objetivo de este estudio fue describir las características de la historia de vida de una población de manantial, *Eurycea guttolineata* (Plethodontidae), para obtener un mejor entendimiento en la evolución de la historia de vida de Plethodontidae. Se encontró que la metamorfosis típicamente ocurre en junio, con un tamaño de 23.08 mm SVL, a una edad de 4-6 meses. El tamaño en la primera reproducción, ≥ 50.00 mm SVL, fue similar entre machos y hembras a una edad de 22-24 meses. Sin embargo, un gran variabilidad en tamaño en hembras sexulamente maduras (2 veces la variabilidad en machos) sugiere que algunas no lleguen a su madurez sexual hasta los 34-36 meses de edad. Los datos sugieren un período de actividad sexual del final del verano hasta el comienzo del invierno (julio a diciembre), con deposición de huevos al comienzo del invierno (noviembre-diciembre), y su eclosión en enero o febrero. Tasas de crecimiento fueron altas durante el primer (2.48 mm SVL/mes) y segundo (1.70 mm SVL/mes) años de vida, mientras que decrecieron (0.31 mm SVL/mes) una vez alacanzada la madurez sexual.

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Adaptation aux particularités climatiques du cycle biologique d'un anoure tropical, *Nectophrynoides occidentalis* Angel, 1943 (Bufonidae)

Maxime LAMOTTE & Coralia SANCHEZ-LAMOTTE

Laboratoire des Reptiles & Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France

In the distribution area of the viviparous toad *Nectophrynoides occidentalis* Angel, 1943, restricted to a few square kilometers of a low grass savanna above 1200 m on the crests of the Mount Nimba, a very dry season of about 5 months alternates with a rainy season of 7 months. The life and breeding cycles of this species are closely linked with this seasonal cycle. During the dry season, the toads burrow underground and become dormant. They emerge as soon as the rains start, between February and the end of March, in the following order : first the gravid females, then the virgin females and finally the males. All births of young take place in June. Fertilization takes place from September for females older than one year, to the end of October for females of that year. All females burrow immediately after fertilization. The cycle does not seem to be modified by the amount of water available in the year, which may vary by twice as much according to the place in the chain or to the year. However, monitoring of the climatic cycle and of toad populations over several years have shown that the dates of burrowing and of dormancy are closely linked to the beginning and above all to the end of the rainy season, that may vary more than one month from year to year. These variations result in important differences in the proportion of young females that are virgin before their first burrowing for the dry season. They have therefore consequences for the reproduction rate of the population.

Nectophrynoides occidentalis Angel, 1943 est un petit amphibien anoure de la famille des Bufonidae dont la longueur museau-anus dépasse rarement 24 mm chez les mâles et 27 mm chez les femelles (fig.1). La coloration est d'un brun ocre chez les mâles, nettement plus claire chez les femelles (ANGEL, 1943, ANGEL & LAMOTTE, 1944, 1948).

L'espèce *N occidentalis* ne vit que sur les quelques kilomètres carrés de la prairie d'altitude (savane à herbes basses) couvrant les crêtes de la chaîne du Nimba dans sa partie située en Guinée et Côte d'Ivoire près de la frontière du Libéria (fig. 2). Présente jusqu'au sommet à 1750 m, elle ne descend pratiquement pas au-dessous de 1200 m d'altitude. Cette localisation très stricte est liée à deux caractéristiques très particulières du milieu.

La première est un relief abrupt (LAMOTTE & ROUGERIE, 1955). Celui-ci exclut presque totalement la présence de mares permettant la vie de têtards et élimine ainsi la concurrence de la presque totalité des autres amphibiens. *Nectophrynoides occidentalis*, en revanche, a pu

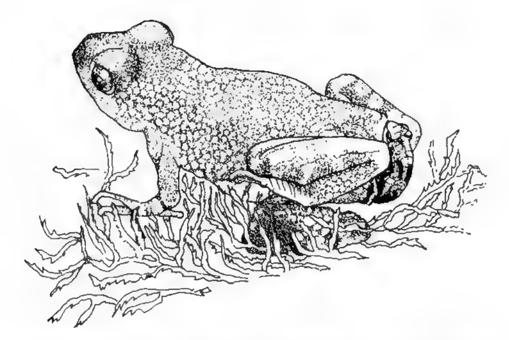


Fig 1 – Aspect d'une femelle de *Nectophrynoides occidentalis* mettant bas un nouveau-né Dessin de Y Schach-Duc d'après une photographie de F. Xavier.

répondre à ce défi par un développement direct dans les oviductes conduisant à la naissance de jeunes entièrement métamorphosés, longs de moins de 8 mm. La figure 3 représente les principaux stades de ce développement, qui dure près de 9 mois (Angel & Lamotte, 1944, 1948; Lamotte, 1959; Lamotte & Xavier, 1972).

En second lieu, les conditions climatiques font alterner une saison des pluies très favorable durant laquelle règne en permanence une forte humidité de l'atmosphère et une saison sèche particulièrement rigoureuse où le degré hygrométrique s'abaisse souvent au dessous de 30 % (Richard-Molard et al., 1955) (fig 4). L'espèce répond à ce contraste climatique accentué par un cycle biologique déterminé lui-même avec rigueur.

Le cycle saisonnier moyen des populations

Durant la saison pluvieuse, l'humidité persistante du milieu liée aux précipitations, aux bruines et aux brouillards lui permet de maintenir son activité de façon ininterrompue, tandis que pendant la saison sèche aucun amphibien ne peut survivre autrement qu'enfoui dans le sol, ce que fait effectivement *Nectophrynoides* en mettant à profit des fissures de la roche sous-jacente.

Des prélèvements quantitatifs effectués sur des surfaces de 25 m^2 en divers sites de la chaîne et au cours des mois successifs de plusieurs années ont permis de suivre les variations de la densité et de la composition des populations. Ils étaient complétés par une étude de la

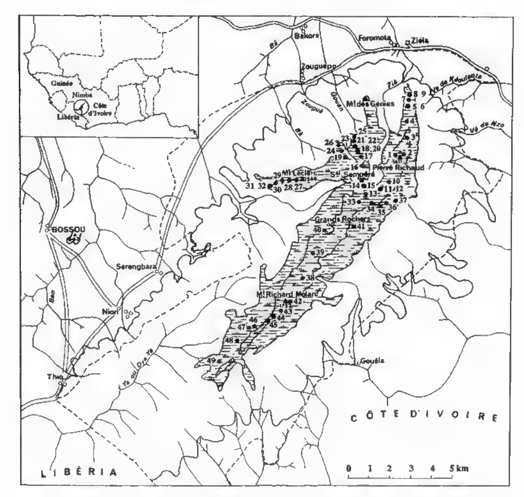


Fig 2 – Situation et relief de la chaîne du Nimba On a figuré les courbes de niveau 600, 1000 et 1400 m, la zone située au dessus de 1000 m est représentée avec un figuré de tirets horizontaux Les nombres indiquent les emplacements où ont été réalisées des collectes du peuplement animal.

taille des individus (liée de façon directe à leur âge) et par la détermination de l'étape de la vie sexuelle des femelles (vierges, gravides, après l'accouchement) qu'indique l'état des oviductes et des ovaires.

Une caractéristique essentielle du cycle biologique est le fait que toutes les naissances se produisent durant le mois de juin, en pleine période de vie active. Encore nettement distincte par sa taille plus petite (de 7 à 13 mm), une nouvelle cohorte vient alors se joindre aux deux plus anciennes (fig 5). Ces femelles adultes, alors âgées de 12-15 mois à 2 ou 3 ans, renferment des individus encore vierges et d'autres qui viennent d'accoucher ; leur taille est de 17 à 28 mm tandis que les mâles des mêmes cohortes mesurent de 14 a 21-22 mm. La population renferme alors un nombre sensiblement égal de mâles et de femelles, et cette égalité plus ou moins

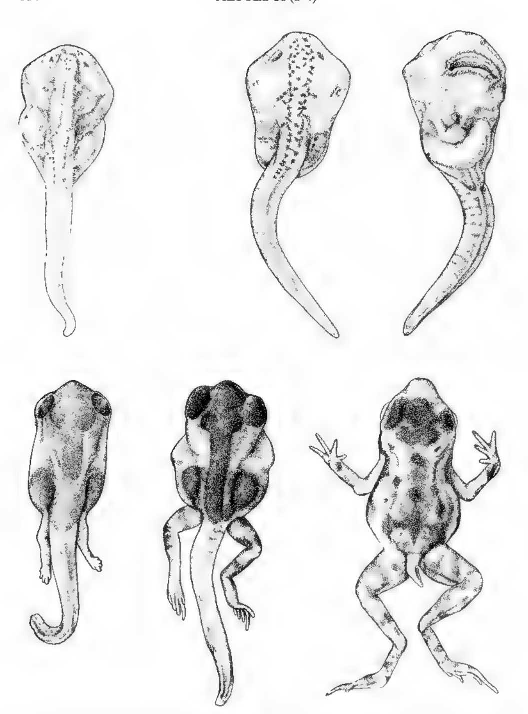


Fig. 3 – Quelques stades du developpement embryonnaire de *N occidentalis* (d'apres LAMOTTE & XAVIER, 1972). Dessins de Y. SCHACH-DUC.

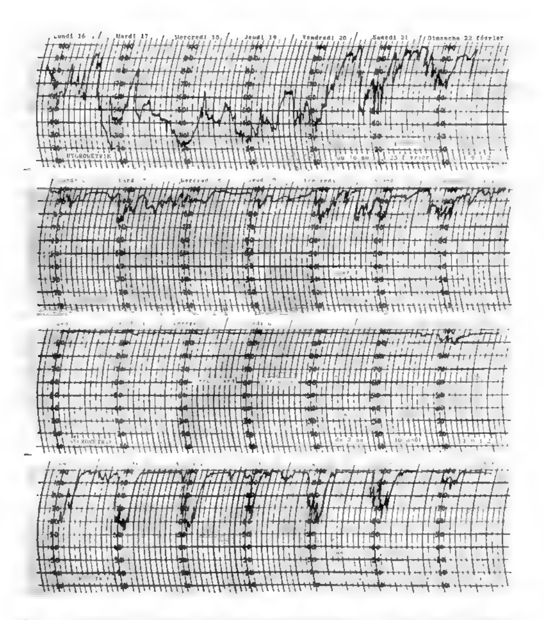


Fig. 4. — Les variations journalières du degré hygrométrique de l'air dans la prairie d'altitude du Nimba à 1600 m. Le degré hygrométrique apporte l'indication la plus adéquate sur les conditions plus ou moins favorables du milieu pour un amphibien. De haut en bas: du 16 au 22 février (saison sèche), du 6 au 12 avril (première saison des tornades), du 3 au 9 août (pleine saison des pluies) et du 15 au 21 octobre (seconde saison des tornades).

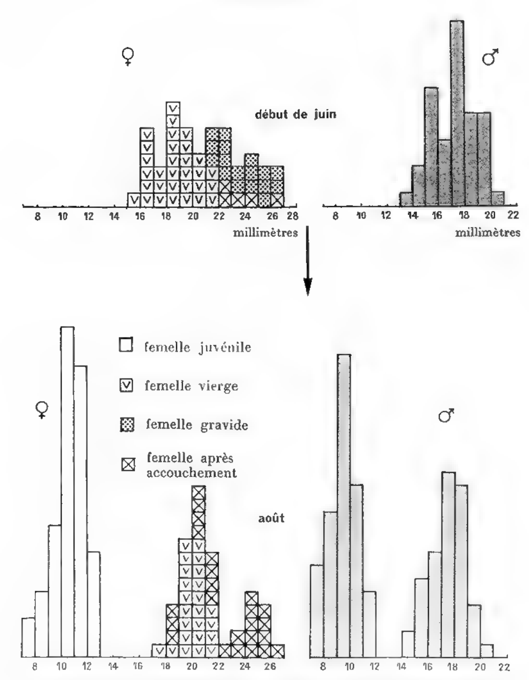


Fig 5 – Composition de la population (femelles et mâles) au début du mois de septembre Apres la période des naissances (en juin), les mois de juillet, août et septembre voient une croissance active de tous les individus. La cohorte des jeunes de l'annee, alors âgés de 1 à 4 mois, se distingue par sa taille nettement plus petite (entre 7 et 14 mm) Il apparaît en outre chez les femelles plus vieilles une coexistence de deux cohortes (respectivement âgees d'environ 16 et 28 mois)

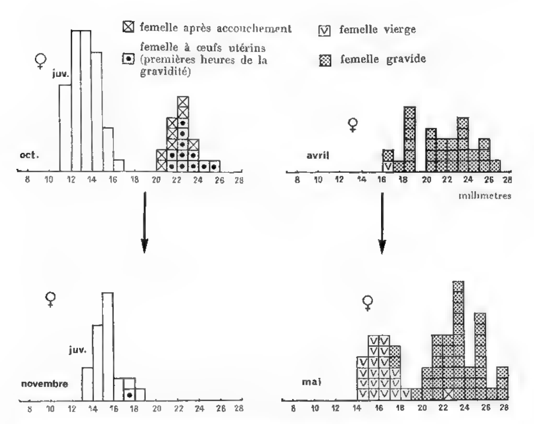


Fig. 6. – Évolution apparente de la population des femelles durant la période d'enfouissement (à gauche) et durant la période d'émergence (à droite).

complète des effectifs des deux sexes persiste durant toute la saison des pluies jusqu'en septembre, tant chez les juvéniles de l'année que chez les individus plus âgés (voir fig. 5).

En fin septembre, avant la fin des pluies, commence la période d'enfouissement qui va permettre à l'espèce de résister à la saison sèche ; cet enfouissement s'étale sur plusieurs semaines. Dès le mois de septembre, période pourtant encore très pluvieuse, les femelles de grande taille, dont c'est la seconde ou la troisième gravidité, s'enfouissent dès qu'elles sont fécondées. Les mâles, au contraire, et surtout les plus jeunes, attendront la fin de la saison des pluies, qui survient en général durant le mois d'octobre. C'est le cas aussi des jeunes femelles vierges, nées 4 mois auparavant ; elles quittent la vie active épigée au fur et à mesure de leur fécondation, elle-même liée à leur degré de développement (fig. 6, à gauche). Les jeunes femelles qui, lorsque cessent les pluies, n'ont pas atteint une maturité suffisante, s'enfouissent encore vierges. Il résulte de ces décalages qu'en octobre et novembre la population de *N. occidentalis* ne comprend plus que des mâles et des femelles vierges nés dans l'année.

La fin de la période de vie enfouie coïncide avec l'arrivée des pluies qui se produit généralement en fin mars, plus exceptionnellement en avril ou en février. Les divers individus

de la population ne sortent toutefois pas tous en même temps, mais avec un décalage qui s'étale sur près d'un mois. Les femelles gravides émergent en premier, puis les mâles et les femelles vierges nées 9 mois auparavant (fig. 6, à droite). Au tout début de la saison des pluies, la population active ne comprend que des femelles gravides (Angel & Lamotte, 1944 ; Lamotte, 1959) ; elles ne sont rejointes qu'ensuite par des femelles vierges et les mâles.

Les conséquences des variations interannuelles du climat sur le cycle des populations

La présentation des traits généraux du cycle des populations en a fait apparaître la liaison étroite avec les variations saisonnières de la pluviosité. Cette dépendance très stricte de la vie de *N. occidentalis* vis-à-vis des facteurs climatiques donne à penser que toute variation de ces facteurs se traduira sur la biologie de l'espèce et notamment sur son cycle de reproduction. Or de telles variations du climat se produisent inévitablement au cours des années successives et des différences existent aussi dans l'espace entre les divers sites de la chaîne où l'espèce est présente.

La hauteur totale des précipitations annuelles est sans doute un facteur important de la localisation de l'espèce puisque celle-ci est absente dans la partie septentrionale de la chaîne où les pluies sont inférieures à 1500 mm. Elle est aussi très variable au sein de l'aire de répartition puisqu'il tombe plus de 3000 mm d'eau au sud du mont Richard-Molard et seulement 2000 mm dans la région septentrionale du Signal Sempéré et du mont Tô. Les différences interannuelles de la pluviosité en un même site de la chaîne sont également très fortes : à la station météorologique de Ziéla, la pluviosité annuelle a varié entre 1099 mm et 1757 mm durant les années 1949 à 1957. Il est toutefois difficile de détecter une influence de cette hauteur annuelle des pluies sur la fécondité de l'espèce qui reste apparemment semblable d'un bout à l'autre de son aire de répartition. Elle est masquée en effet par les variations considérables liées à la taille de la femelle. De fait, les jeunes femelles de moins de 21 mm de longueur museau-anus – fécondées à l'âge de 4 mois – ne portent généralement que de 2 à 8 embryons, tandis que les femelles plus âgées, dont la taille dépasse 22 mm, en ont généralement plus de 10 (fig. 7). Cette relation entre le nombre d'embryons et la taille de la mère se retrouve dans tous les sites de la montagne et toutes les années.

Si la pluviosité annuelle ne semble pas être un facteur majeur du cycle biologique, tout autre est le rôle du calendrier des pluies.

Au cours d'une même année, les dates d'émergence et d'enfouissement sont, comme celles de l'arrivée et de la fin des pluies, sensiblement les mêmes dans toute l'aire de répartition de l'espèce, depuis le Signal Sempéré jusqu'au sud du mont Richard-Molard. Au contraire, ces dates du commencement et de la fin de la période pluvieuse sont très variables d'une année à l'autre et elles déterminent toujours avec rigueur celles de l'émergence et de l'enfouissement des *Nectophrynoides*. Il est ainsi des années où les pluies précoces provoquent une sortie des crapauds dès la fin de février et d'autres où les pluies, et avec elles l'émergence, n'arrivent que fin avril ou début mai. Inversement, la fin de la période des pluies et donc celle de la vie active des derniers individus – jeunes femelles non fécondées et mâles parmi lesquels dominent des jeunes de l'année – peuvent se produire dès le début du mois d'octobre ou au contraire au début novembre, voire en décembre.

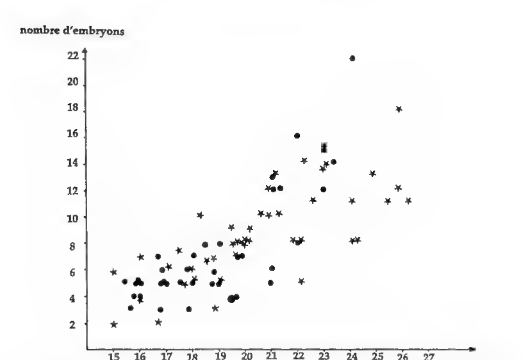


FIG 7 Nombre d'embryons en fonction de la taille de la mère (longueur museau-anus). Les points et les etoiles correspondent respectivement aux annees 1981 et 1991.

Une analyse plus précise de la structure démographique des populations poursuivie durant plusieurs années conjointement avec des enregistrements pluviométriques mensuels a permis de pousser plus loin l'étude de l'influence du cycle saisonnier des pluies (LAMOTTE, 1959). Elle a fait apparaître une corrélation nette entre la pluviosité des mois d'octobre et novembre et la proportion dans la population de jeunes femelles restées vierges parce qu'immatures lors de l'enfouissement à l'arrivée de la saison seche (fig. 8). Une venue précoce de la saison sèche, dès le début octobre, diminue ainsi la participation de la cohorte de jeunes femelles de l'année au renouvellement de la population, tandis que le prolongement de la saison des pluies permet le développement jusqu'à leur maturité de la majorité de ces individus.

Les femelles plus âgees, elles, sont toutes fécondées dès le mois de septembre et fournissent donc toutes, quelle que soit la date de la fin des pluies, le même contingent d'embryons. Durant les années à saison seche précoce, la contribution à la natalité de la cohorte des jeunes de l'année peut ainsi tomber à 7 % seulement, alors qu'elle représente jusqu'à 25 % quand la saison des pluies se prolonge jusqu'en fin novembre. C'est dire l'influence considérable qu'auraient plusieurs années défavorables consécutives sur la démographie de l'espèce.

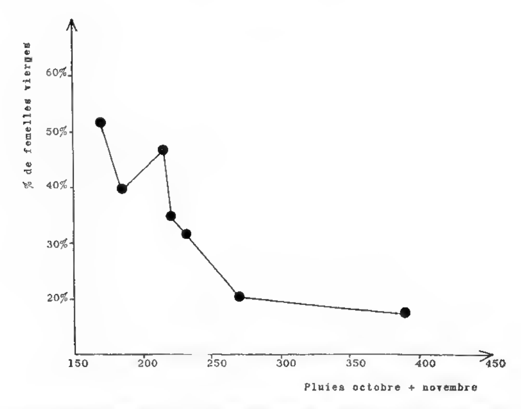


Fig 8. Variation interannuelle du pourcentage des femelles restées vierges lors de leur enfouissement en fonction des *quantités de pluie* tombées en *octobre et novembre de l'année de leur naissance* (d'apres Lamotte, 1959).

Comme le montre la fig. 9, la pluviosité annuelle totale est, contrairement à la fin plus ou moins précoce de la saison des pluies, sans action sur la proportion de femelles restant vierges avant l'enfouissement.

Conclusions

Les études menées sur le terrain entre 1942 et 1991 ont fait apparaître l'étroite corrélation qui existe entre les populations du petit bufonidé vivipare orobionte *Nectophrynoides occidentalis* et le cycle climatique de la prairie d'altitude où il est localisé. La corrélation, qui se manifeste déjà avec rigueur à l'échelle de l'année climatique moyenne, est coroborée et précisée par la comparaison de plusieurs annees différant par leur cycle saisonnier Celle-ci fait ressortir le rôle prépondérant du calendrier des pluies et plus particulièrement de l'arrivée plus ou moins précoce de la saison sèche qui influe sur le pourcentage de jeunes femelles de l'année fécondees avant de s'enfouir La fécondité globale de l'espèce peut être ainsi considérablement modifiée.



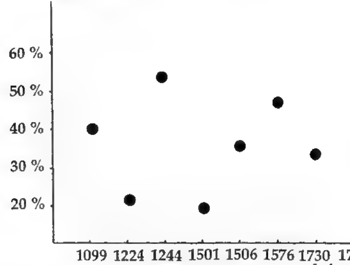


Fig. 9 Pourcentage de femelles restées vierges lors de leur enfouissement en fonction de la *pluviosité totale (en mm) de l'année de leur naissance.*

RÉSUMÉ

Dans l'aire de répartition du crapaud vivipare *Nectophrynoides occidentalis* Angel, 1943, limitée à quelques kilomètres carrés d'une savane à herbes courtes couvrant les crêtes du mont Nimba au-dessus de 1200 m d'altitude, le climat est caractérisé par l'alternance d'une saison très sèche de l'ordre de 5 mois contrastant avec une saison de 7 mois de pluies et de bruines.

Les cycles de vie et de reproduction de l'espèce sont étroitement liés à ce cycle saisonnier. Durant la saison sèche, les crapauds sont enfouis dans le sol en état de vie ralentie. Ils sortent dès l'apparition des pluies, entre février et fin mars, avec un décalage entre les femelles gravides, qui sortent les premières, les femelles vierges et enfin les mâles. Toutes les mises-bas ont lieu en juin. Les fécondations se font en septembre pour les femelles âgées de plus d'un an, jusqu'en fin octobre pour les femelles de l'année. Toutes s'enfouissent aussitôt fécondées.

Le cycle ne semble pas modifié par la quantité d'eau tombée annuellement, pourtant variable du simple au double selon l'emplacement dans la chaîne et selon l'année. En revanche, le suivi du cycle climatique et celui des populations au cours de plusieurs années a montré que les dates de l'enfouissement et de la sortie de la vie ralentie sont liées étroitement à l'arrivée et surtout a la fin de la saison des pluie qui peuvent différer de plus d'un mois selon les années. Ces variations se traduisent par des différences importantes de la proportion de jeunes femelles restees vierges avant de s'enfouir pour leur premiere saison seche. Elles se répercutent ainsi sur la fécondité globale de la population

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Notes on morphological variation and the biology of *Nototriton guanacaste* Good & Wake, 1993 (Caudata, Plethodontidae)

Michael FRANZEN

Zoologische Staatssammlung, Münchhausenstr 21, 81247 München, Germany

The variation in body size, body proportions, and coloration of *Nototriton guanacaste* Good & Wake, 1993 is greater than documented previously. Data from seven newly collected specimens suggest that the character "snout-gular length", previously considered to be diagnostic, widely overlaps with that of other Costa Rican *Nototriton* species. The variation of some aspects of coloration is considerably greater than in the type series. Regarding the habitat, *N. guanacaste* seems to prefer locations among roots of epiphytes growing in moss mats.

INTRODUCTION

Despite the comprehensive study of GOOD & WAKE (1993), the diminutive and inconspicuous plethodontid salamanders of the genus *Nototriton* are among the least known species of the Costa Rican amphibian fauna. I collected specimens of the recently described *Nototriton guanacaste* Good & Wake, 1993, which is endemic to two isolated peaks in northwestern Costa Rica. This material provides new information on morphological variation with respect to body size, body proportions, coloration and on habitat and biology

MATERIAL AND METHODS

Specimens of *Nototriton guanacaste* here studied are deposited in the collection of the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK). Seven specimens and two clutches were collected at the type locality in Guanacaste National Park, Costa Rica, in the western summit area of Cerro Cacao (1450-1550 m) on 7 September 1993 (ZFMK 67726), 13 September 1993 (ZFMK 67728) and 23 September 1993 (ZFMK 67727, 57729-57732). Furthermore, the material consists of a clutch of eight eggs deposited on 7 September 1993 (three preserved on 7 September 1993: ZFMK 57733; five preserved on 12 October 1993: ZFMK 57734) and a clutch of five eggs found on 23 September 1993 (one

preserved on 23 September 1993. ZFMK 57735; four preserved on 12 October 1993. ZFMK 57736). Measurements follow the standards defined by Brame (1968), and were made under a dissecting microscope fitted with an ocular micrometer.

Results

Morphology

Measurements and morphometric dimensions of the newly collected material are shown in tab. 1. Most of the newly ascertained body measurements and proportions (axilla-groin length, head width, nostril diameter, forelimb length, hindlimb length, foot width, third and fith toe length) agree well or are at least very near to those of the type series. However, maximum length and variation of some body proportions are larger than previously documented. Three of the seven newly collected specimens have larger snout-vent lengths than the largest specimens of the type series (holotype. 29.7 mm). Furthermore relative trunk width is consistently larger in the newly collected material, with no overlap with the range of the type series. In contrast, relative tail length is shorter in the newly collected material, likewise with no overlap with the range of the type series. With respect to the relative snout-gular length ("head length"), only the two smallest specimens are near to measurements of the type specimens, with head lengths of 20 3 and 21 3 percent of snout-vent length. Relative snout-gular length is strongly negatively correlated with snout-vent length ($r = -0.88$; $P = 0.004$). Regarding the development of the parotoid glands, only a somewhat physically enlarged parotoid region is discernible in all specimens of the new material.

The preserved specimens are shown in fig. 1. Two specimens (ZFMK 57726 and 57732) show a conspicuous bright dorsal ground coloration, which was light brownish-orange in life. Within the entire series, the lateral and dorsal ground color varied from light brownish-orange to dark brown in life. One of the seven new specimens (ZFMK 57731) has a bright lateral coloration In two specimens (ZFMK 57727 and 57729), the flanks are slightly brighter than the dark brown dorsum, whereas the four remaining specimens have a lateral coloration which is identical (ZFMK 57728) or darker than the dorsal ground color. The bright coloration of the parotoid region is evident in all the new specimens. However, a bright elongate blotch on the parotoids is indistinct and very small in ZFMK 57728 and 57730. In ZFMK 57726 and 57732, parotoid glands cannot be discerned by their color due to an overall bright dorsal coloration

Notes on biology

All specimens of *Nototriton guanacaste* were observed in 10-20 cm thick dripping wet moss mats growing on trees in "lower montane rain forest" (sensu Tosi, 1969 common names, "cloud forest", "elfin forest") near the summit of Cerro Cacao. During 12 hours of searching, three salamanders were taken from moss clumps hanging from twigs and branches, whereas four specimens were found within 30 minutes on horizontal branches among the

Table 1 – Measurements (mm), followed in parentheses by morphometric ratios (percent of snout-vent length), of the seven newly collected *Nototriton guanacaste* specimens compared with the range of the type series (after GOOD & WAKE 1993) SVL: snout-vent length.

	ZFMK 57727	ZFMK 57729	ZFMK 57726	ZFMK 57728	ZFMK 57731	ZFMK 57730	ZFMK 57732	Range % SVL (hoc loco)	Range % SVL (type series)
Sex	male	male	female	female	female	cf. female	cf female		
Snout-vent length	30.9	26.8	33.5	33.0	27.5	22.2	22.1	–	–
Axilla-groin length	17.7 (57.3)	14.0 (52 2)	20.0 (60 6)	20 0 (59 7)	15.7 (57.1)	12 0 (54 1)	12.3 (55.7)	52.2 – 60.6	54.5 56.3
Trunk width	4 7 (15.2)	4.0 (14.9)	4 5 (13.6)	5.0 (14.9)	4.0 (14.5)	3.2 (14.4)	3.3 (14.9)	13 6 – 15.2	11.0 12.2
Tail length	–	–	39.0 (116.4)	–	28.9 (105.1)	24.6 (110.8)	23.9 (108.1)	105.1 116.4	121 0 133 7
Snout-gular length	5 8 (18 8)	5 2 (19.4)	6.1 (18 5)	5.9 (17.6)	5.0 (18.2)	4.5 (20 3)	4.7 (21.3)	17.6 21.3	21.6 – 22.4
Head width	4 1 (13 3)	3.9 (14.6)	4.2 (12.7)	4.4 (13 1)	3.8 (13.8)	3.3 (14.9)	3.5 (15.8)	12.7 – 15 8	14.5 – 15.7
Nostril diameter	0 24 (0.78)	0.19 (0.71)	0.12 (0 36)	0 19 (0 57)	0.17 (0.62)	0 21 (0 95)	0.21 (0.95)	0 36 0 95	0 4 0.9
Forelimb length	5 8 (18 8)	4 6 (17 2)	5 6 (17.0)	5.3 (15.8)	4 2 (15 3)	3.8 (17.1)	3 9 (17 6)	15 3 – 18.8	17 0 – 17.9
Hindlimb length	6 4 (20 7)	5 3 (19 8)	5 8 (17 6)	5.8 (17.3)	4 8 (17 5)	4.4 (19.8)	4 4 (19.9)	17.3 – 20.7	18.5 – 20.1
Foot width	2 5 (8 1)	2 0 (7 5)	2 3 (7 0)	2.1 (6 3)	1 9 (6 9)	1.3 (5 9)	1 5 (6.8)	5.9 - 8 1	6.6 – 7.2
Third toe length	1 0 (3.2)	0.9 (3.4)	1 2 (3 6)	0 9 (2.7)	0 8 (2 9)	0 7 (3.2)	0.6 (2.7)	2.7 – 3.6	2.8 3.1
Fifth toe length	0 6 (1.9)	0.4 (1.5)	0 6 (1.8)	0 5 (1 5)	0.5 (1.8)	0 3 (1.4)	0 3 (1 4)	1.4 – 1.8	1.1 – 1.7



Fig. 1. – Variation in coloration in *Nototriton guanacaste* from Cerro Cacao (Guanacaste, Costa Rica). Photo: Juliane POPP.

roots of epiphytes growing in the moss mats. In such microhabitats, the habitus of the salamanders was remarkably similar to that of epiphytic roots. All animals were found 0.5 to 5 meters above the ground. Substrate temperatures ranged from 18.6 to 21.5°C.

When grasped, all salamanders showed "coil-uncoil flip" and "running flip" defensive behaviors (DODD & BRODIE, 1976). Flips were observed as far as 50 cm.

On 7 September 1993, female ZFMK 57726 and two single eggs were taken from a moss clump growing on an air root two meters above the ground. The night after capture, the female deposited six eggs in the moss of the transportation container. An unguarded clutch of five eggs, containing well developed embryos, was taken on 23 September 1993 from a moss mat

growing on a vertical tree trunk about two meters above the ground. Both clutches were stored in wet moss at room temperature in the laboratory for a time. Most eggs of both clutches developed well until they were perserved.

DISCUSSION

Most of the here ascertained differences in morphometric dimensions are not surprising, since only five individuals of *N. guanacaste* were analyzed in the original description by GOOD & WAKE (1993) The data on the snout-vent lengths of the new specimens suggest that those of the type series are not fully grown, though obviously mature (according to GOOD & WAKE, 1993: 138, one male specimen of the type series has a "rather flat and inconspicuous mental gland"). The new ascertained maximum snout-vent length of 33.5 mm (female, ZFMK 57726) makes *N. guanacaste* the second largest among the Costa Rican *Nototriton* species. Only the single known specimen of *N major* Good & Wake, 1993 has a larger size with a snout-vent length of 37 9 mm. Differences in relative tail lengths of the new specimens compared to the types may be caused by slightly different measurements. I measured snout-vent length from the anterior tip of the snout to the posterior angle of vent. If measured to the anterior angle of vent (and subsequently tail length from anterior angle to the tip of the tail), the new specimens have relative tail lengths of 112.8 to 128.9 (mean 122.5 ± 7.12) percent of snout-vent length This is well within the range of the data given in the original description. Relative snout-gular length is a major diagnostic feature which separates *N. guanacaste* from all other Costa Rican *Nototriton* (GOOD & WAKE, 1993). The revised range of 17.6 to 22.4 percent in this character (including data from GOOD & WAKE, 1993) widely overlaps with *N picadoi* (Stejneger, 1911), *N. richardi* (Taylor, 1949), *N. tapanti* Good & Wake, 1993, *N major* Good & Wake, 1993 and various populations of *N. abscondens* (Taylor, 1948). The differences between my own data and those of GOOD & WAKE (1993) can be explained by the smaller size of the type specimens (see above): relative snout-gular length is significantly negatively correlated with snout-vent length. In other words, smaller animals have longer heads and head length shows a changing relationship to body size as animals grow. Another diagnostic feature which separates *N. guanacaste* from *N abscondens* according to GOOD & WAKE (1993) is its prominent parotoid glands. I ascertained only rather flat and inconspicuous parotoid regions in the new material However, it is relative to some degree to regard a character as "prominent" or "indistinct", and the difference may be caused by my limited experience with other *Nototriton* species. A single specimen of *N. abscondens* (El Angel Waterfall, Provincia de Alajuela, Costa Rica, in my private collection) indeed shows much more reduced, almost invisible parotoid glands.

The robust habitus (as measured by "trunk width") of *N. guanacaste*, that makes it unmistakable among Costa Rican species, is confirmed by the newly collected material. The revised range with a maximum of 15.2 percent of snout-vent length even emphasizes differences to the other species. However, one should keep in mind that differences between the new material and the type series may be caused by different methods of conservation.

All in all the robust habitus and the confirmed small nostril diameter (which is a major character separating *N guanacaste* from the geographically nearest population of

N. abscondens at Monteverde) support the specific status of *N guanacaste* from the morphological point of view.

Variation in coloration of the newly collected specimens is considerably greater than in the type series. A bright lateral coloration, as reported in previously collected specimens, is evident in only one specimen. Bright parotoid blotches are indistinct and very small in two specimens. Good & Wake (1993) mentioned that these markings were less evident in their smaller specimens. However, markings are inconspicuous among the new material in one large (ZFMK 57728) and one small specimen (ZFMK 57730).

The observations regarding the biology agree well with data known for *N. guanacaste* and other *Nototriton* species. Like all previously observed specimens, the new material was found in moss mats on trees above the ground. Regarding the microhabitat, the new specimens were observed with different success in two different structures. in moss clumps hanging from air roots or growing on vertical branches (0.25 specimen/hour) and in moss mats among roots of epiphytic ferns and bromeliads on horizontal branches (8 specimens/hour). Due to the small number of observed specimens, these results may be accidental. Nevertheless, it can be considered that humidity conditions are more stable in the latter microhabitat due to a higher proportion of humus and an overall thicker and more compact substrate cover.

The defensive behaviors "coil-uncoil flip" and "running flip" were previously reported by Dodd & Brodie (1976) for other neotropical plethodontids, including "*Chiropterotriton picadoi*" (i.e., *Nototriton richardi* or *N. abscondens* sensu Good & Wake, 1993) I observed that juvenile and adult *N picadoi* and *N abscondens* show the same behaviors in the field and in captivity.

The clutch sizes of five and eight eggs observed during the present study correspond to the data given by Good & Wake (1993). two clutches with four and seven eggs. In other *Nototriton* species, clutch size may be as high as 17 eggs (Jokusch & Garcia-Paris, 1998).

Nototriton and *Oedipina* are presumed to be the only bolitoglossines which abandon their clutches (Good & Wake, 1993). The finding of another unguarded clutch of *N. guanacaste* supports this to some degree. It should be noted that I also found two further unguarded clutches (with two and three eggs) of unidentified *Nototriton* on 3 and 4 October 1993 at Tapanti, Costa Rica.

Though considerably different to the type series in some aspects, I regard the newly collected material as belonging to a single species. Differences in morphometric dimensions are consistent among the newly collected material (tail length) or vary gradually (snout-gular length). Furthermore, differences in coloration (parotoids and flanks) are not associated with differences in body proportions or snout-vent length. *Nototriton* species can be highly specific to microhabitats (see Campbell & Smith, 1998), so the different microhabitats observed in the present study may give a hint for a specific differentiation However, the occurrence of color morphs (e.g., animals with dark flanks or animals with a bright overall coloration) did not correspond to a certain type of microhabitat.

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Limits of the morphometric method for field identification of water frogs

Alain Pagano & Pierre Joly

ESA CNRS 5023 Ecologie des eaux douces et des grands fleuves,
Université C. Bernard Lyon 1, 69622 Villeurbanne Cédex, France
e-mail: Pagano@univ-lyon1.fr, pjoly@biomserv univ-lyon1.fr

Taxonomic identification of the water frogs has evolved since hybridogenesis has been revealed within the *Rana esculenta* complex. Although the study of protein polymorphism has proved robust in taxonomic information, morphometric measurements are currently used despite of some limitations of the method. By comparing results obtained with these two techniques, this study shows that morphometry is not always decisive for field identification. In the three populations studied, in the mid-Rhône floodplain, the morphs of *Rana ridibunda* and the hybrid *Rana* kl. *esculenta* greatly overlap in morphometric characters.

Introduction

The Palearctic water frog group is composed of several species (for a review see Dubois & Ohler, 1995) and is characterized by three hybridogenetic complexes (synkleptons sensu Polls-Pelaz, 1989). The *Rana esculenta* complex, which is widespread in central Europe, is the more studied of these complexes. The three taxa of this synklepton (*Rana ridibunda*, *Rana lessonae* and the hybridogenetic hybrid *Rana* kl *esculenta*) have been distinguished by several morphological characters for a long time (e.g. Camerano, 1884), but the systematics of water frogs remained confused until the existence of a hybrid complex was demonstrated (Berger, 1968) In this context, the morphometric indices proposed by Berger (1966) to discriminate three morphs among the hybridogenetic complex strongly contributed to the systematics of the group, and this method is still commonly used (for a recent review, see Ogielska, 1995).

Nevertheless, several morphometric investigations showed an overlap among the characteristic morphs of several taxa (e.g. Günther et al., 1991; Polls-Pelaz, 1991; Rybacki, 1995). Besides using the morphological indices proposed by Berger (1966), some authors applied sophisticated analysis (discriminant analysis, multivariate analysis) to maximize the morphological differences between taxa (e.g. Uzzell & Hotz, 1979, Plötner et al., 1994). Despite the increasing complexity of taxonomic identification on the basis of morphometric variables, this morphometric method still remains. On the other hand, the analysis of protein polymorphism proves robust in taxonomic identification.

Although the use of quantitative morphological traits fails in identification of water frogs in eastern France (Joly et al., 1995, Tunner, personal communication), some studies

only used the morphometric method in frog taxonomy. Because of large number of individuals to be identified, field studies need simple methods. In this context, the aim of this paper was to compare the simplest morphometric measurements currently used (e.g. Dp/Cint) with the analysis of allozymic markers.

MATERIAL AND METHODS

SITES AND SAMPLE SIZES

Three populations (Morte-de-la-Barre, Jons, Pierre-Bénite) were investigated in sites located near the active channel of the Rhône river. The former two ponds are gravel-pits while the last one is a regularly overflowed side arm of the Rhône. The sample size is the following: Pierre-Bénite, $n = 28$ (15 males and 13 females); Jons, $n = 31$ (19 males and 12 females); Morte-de-la-Barre, $n = 33$ (25 males and 8 females). Voucher numbers are: Jo26-33, Jo35, Jo37-38, Jo40-47, Jo55, Jo92-102, PB50-54, PB103-125, MB56-63, MB65-71, MB74-91, all deep-frozen carcasses, kept in our laboratory (Université Lyon 1, France).

PROTEIN ELECTROPHORESIS

Electrophoresis was performed on skeletal muscles. Tissue samples were crushed in a 1.2 g Tris + 0.37 g EDTA + 1 l H_2O + 50 ml NADP 1 % solution. Migration was performed in a Tris citrate gel at pH 6 during 3 to 5 hours under 180 Volts. Tris citrate gel composition was: 48 g starch (12 %), 1.4 ml buffer 1 × (composition of the 10 × buffer: Tris 270 g, citric acid 181 g, H_2O 1000 ml), 398.6 ml H_2O Staining solutions were prepared using modifications of standard procedures (PASTEUR et al., 1987, HOTZ, unpublished).

Four loci were analyzed for somatic tissues: lactate deshydrogenase (LDH-1, Enzyme Commission 1.1.1.27), mannose-phosphate-isomerase (MPI, E C 5.3.1 8), phosphoglucomutase (PGM-2, E C. 2.7.5 1) and creatine kinase (CK, E.C 2.7.3.2). These enzymes were chosen because they are known to be efficient for taxonomic identification of several species and hybrids of water frogs (for review, see HOTZ, 1983 and BEERLI, 1994).

Reference specimens from the collection of the Zürich University (H HOTZ) were used as control samples (2 specimens for each of the following taxa): *Rana perezi* (Elvo Delta, Spain), *Rana* kl. *grafi* (Pouzolles, France), *Rana ridibunda* (Mosina, Poland), *Rana* kl. *esculenta* (Hellberg, Switzerland) and *Rana lessonae* (Poznan, Poland and Hellberg, Switzerland). Respective voucher numbers are. 17027, 17030, 17570, 17572, 18095, 18096, 18011, 18109, 18094, 18102, all deep-frozen tissues (no carcasses), kept in the Zürich University (Switzerland).

MORPHOMETRY

The method of SAGNES (1995) was used in collecting morphometric data Demedulated animals were disposed on a box, near a scale A photograph taken using a video camera was numerized by the computer Using the "Image © software", we scaled the photographs and the variables were measured (fig. 1) Because this software allows to zoom a part of the photograph for measuring variables of small size (the metatarsal tubercle in our study), the

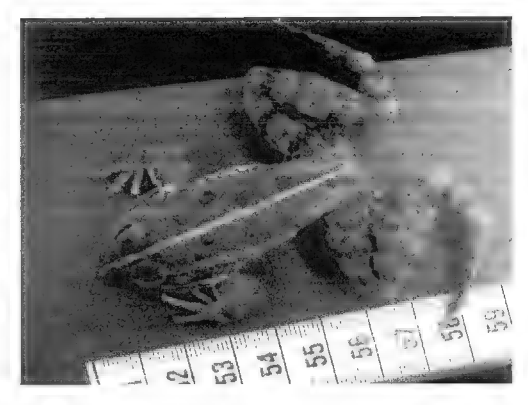


Fig 1. A specimen of water frog numerized and measured by computer software.

errors in measuring parameters were minimized (Sagnes, 1995). Five variables were measured on computerized frog photographs: Lc (body length), Ti (Tibia length), Dp (First toe length) Cint (Metatarsal tubercle length) and Cint-a (Metatarsal tubercle height). These measurements were used to calculate morphometric indices (Dp/Cint, Ti/Cint, Ti/Cint-a) that are known to discriminate the three forms of the *esculenta* synklepton (Berger, 1966). Male and female analyses were done separately. Measurements were made before freezing the animals.

Results

Electrophoretic identification

The analysis of specific markers in the loci studied established the presence of *Rana ridibunda* and *R.* kl *esculenta*, and the absence of *R. lessonae*, *R. perezi* and *R.* kl *grafi* in the sites studied (tab. 1).

Whereas the Jons population was exclusively composed of *R ridibunda*, the others were mixed populations of *R. ridibunda* and *R.* kl. *esculenta* with 12 % and 19 % of hybrids in Morte-de-la-Barre and Pierre-Bénite, respectively.

Table 1 Specific allozymes or specific genotypes which allow taxonomic identification of water frogs.

Allozymes or genotypes				Species	Number of frogs per site		
LDH-B	MPI	PGM-2	CK-A		Pierre Bénite	Jons	Morte Barre
Allozyme a or c	Allozyme a or c	Allozyme b or d	(1)	*Rana ridibunda*	25	31	29
Genotype ae or ce	Genotype ah	Genotype cd	(1)	*Rana* kl. *esculenta*	6	0	4
Allozyme i or d	Allozyme l or m	(2)	Allozyme d	*Rana perezi*	0	0	0

(1) No specific marker between *R. lessonae* and *R. ridibunda*. The identification of *R.* kl. *esculenta* is not possible with only this locus.
(2) No specific marker between *R perezi* and *R. ridibunda*.

MORPHOMETRIC IDENTIFICATION

The graph Dp/Cint versus Ti/Cint usually discriminates the different forms of the *esculenta* synklepton (BERGER, 1966). However, in the populations studied and with the morphometric method used (based on computerized photographs), these morphological indices did not clearly separate the different morphotypes neither for males nor for females (fig. 2). Thus, for males, the use of genetic taxonomic markers revealed that the morphological indices of *R.* kl *esculenta* widely overlapped those of *R ridibunda* in the populations studied (fig. 2), and most of the hybrids could not be distinguished from *R ridibunda* using these indices. Whereas an overlapping was also evidenced for females, the small sample size does not allow a decisive conclusion

DISCUSSION

In central and eastern Europe, each taxon of the *R. esculenta* synklepton can be identified by several morphological indices (BERGER, 1966, BLANKENHORN et al, 1971,

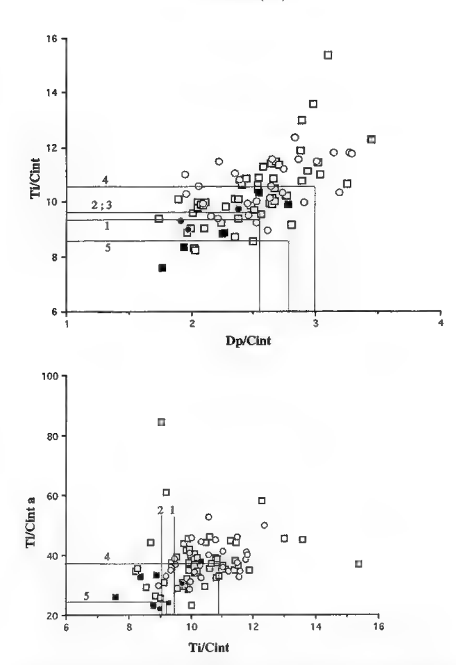


Fig. 2. – The usual graphs Ti/Cint versus Dp/Cint and Ti/Cint versus Ti/Cint-a reveal a great overlapping between the morphotypes of *R.* kl. *esculenta* (black) and *R. ridibunda* (white). Squares symbolize males and circles females. Several thresholds are represented. These limits discriminate *R.* kl. *esculenta* from *R. ridibunda* in the following respective references: (1) BERGER, 1966, (2) COLGANICEANU & TESIO, 1993; (3) POLLS-PELAZ, 1991; (4) RÉGNIER & NEVEU, 1986, (5) WIJNANDS & VAN GELDER, 1976. None of these references make it possible to identify the frogs of the present sample.

WIJNANDS & VAN GELDER, 1976). However, in several studies, morphological identification did not correspond with genetic identification (e.g. GÜNTHER et al., 1991; POLLS-PELAZ, 1991; RYBACKI, 1995) In our study, morphological features of *esculenta* males greatly overlapped with those of *R. ridibunda* and no clear morphotype (as currently described) was detected. Thus the morphometric indices are not always valid for taxonomic identification in the field. Morphometric identification is far from being secure, at least in the studied region and using our method (photographs of non-fixed animals). Other studies evidenced similar problems of taxonomic identification (JOLY et al., 1995; KOTLIC & SULOVA, 1995; LADA et al., 1995; RYBACKI, 1995, MORAND et al., in preparation). Thus, the limitations of identification using these indices are striking when we report the values of Ti/Cint given by several authors as discriminating values for the three morphs of the *R. esculenta* synklepton. Thresholds vary between studies (see tab. 2 for a review and fig. 2). Though it may be argued that there are artefactual differences linked to differences in methods (fixed specimens or living frogs, differences in measurement methods, investigations with or without taking care of morphometric differences between males and females), such a variation in morphological traits suggests several other hypotheses or questions:

(1) Are morphological traits more representative of adaptation than of phylogenetic relationships? Some ecological variables in relation to a gradient of flood disturbance lead to this hypothesis (MORAND et al., in preparation). The sites we studied were within a floodplain where ecological successions are rapid and different habitats patchily distributed. In tadpoles, variation in size is greater in unpredictable environments than in predictable ones (WILBUR & COLLINS, 1973). Morphology is probably determined on the one hand by phylogenetic constraints and on the other hand by environmental conditions. The absence of distinct morphotypes can be explained by the expression of phenotypic diversity in the context of unpredictable and heterogeneous environments. So, we hypothesize that morphological discrimination found in several studies in stable environments is perhaps more an effect of different, separate and stable habitats than the result of phylogenetic lineage. However, there is no evidence in the literature to support this statement because of a lack of ecological description of sites (PAGANO et al., in preparation). Morphometric method was more used as a taxonomic tool than for ecological investigations. In a same taxon, the morphological variation between populations of different biogeographic regions (tab. 2) can be the result of genetic structurations. Several studies have shown that *R. ridibunda* is highly variable (HOTZ et al., 1985; BEERLI, 1994; PAGANO et al., 1997). Besides, the genetic distance between *R.* kl *esculenta* of France and central Europe is unknown. The hypothesis of genetic structuration within a taxon remains to be tested

(2) According to GROSSENBACHER (1988), the presence of *R. ridibunda* in the upper-Rhône river is recent and due to introductions. In this respect, we can hypothesize that, for a long time, *R.* kl. *esculenta* lived alone in habitats favorable for *R. ridibunda*. So its morphology may reflect its adaptation to these habitats. The absence of distinct morphotypes for *R. ridibunda* and *R.* kl. *esculenta* could be explained by convergence.

(3) Does temperature influence morphological variation? REPA (1977) showed that tibia length was related to the mean water temperature of the ponds. The epigenetic origin of morphological variation has to be studied Such an idea has been suggested to explain the high values of indices in water frogs from western France (RÉGNIER & NEVEU, 1986).

Table 2. – Differences in the discriminating values of the index Ti/Cint for the identification of water frogs in some countries of Europe.

References	*Rana lessonae*	*Rana* kl. *esculenta*	*Rana ridibunda*	Country
Régnier & Neveu, 1986	< 9.5	9 - 10.4	–	France (Bretagne, North-East)
Polls-Pelaz, 1991	< 8	8 - 9.5	–	France (Paris region)
Günther, 1975	< 7	6.5 - 8.6	–	Germany
Wijnands & Van Gelder, 1976	< 6	6 - 8.5	> 8.5	Netherlands
Berger, 1966	< 7	7 - 9	> 9.5	Poland
Cogalniceanu & Tesio, 1993	< 7	7 - 9.5	> 9.5	Romania

In several studies, investigations were performed on the basis of the sole morphometric identification, but we assert that such an identification is far from being secure. For the moment, only genetic identification provides decisive criteria for taxonomic identification.

Because several studies (experimentation, field studies, etc) need identification of living animals, we may recommend the use of electrophoresis. It is possible to perform such an analysis on a small piece of tissue (a cut toe or blood; Hotz, personal communication; Pagano, unpublished data), so that data collection is easy in the field. However, other morphological criteria allowing identification may be found, such as the shape of the vomerine teeth (Crochet et al., 1995), though the pertinence of such methods has to be checked by extensive comparison with electrophoretic data.

Résumé

Pour des raisons historiques, la morphométrie est couramment utilisée pour la détermination taxinomique des grenouilles vertes du complexe *Rana esculenta*. L'utilisation de l'éléctrophorèse de protéines est souvent utilisée à des fins identiques. Dans cette étude, la détermination des spécimens a été effectuée à la fois par l'analyse du polymorphisme enzymatique et par la morphométrie en analyse d'images, contribuant à montrer que cette dernière technique n'est pas totalement fiable pour des determinations sur le terrain.

ACKNOWLEDGEMENTS

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Estructura del condrocráneo y esqueleto visceral de larvas de *Pseudis minuta* (Anura, Pseudidae)

E. O. Lavilla* & Rafael O. De Sá**

* Fundación Miguel Lillo, Miguel Lillo 251, 4000 Tucumán, Argentina

** Department of Biology, University of Richmond Richmond, VA 23173, Estados Unidos

The chondrocranium and visceral skeleton of *Pseudis minuta* tadpoles are described, based on a series of five larvae in stages 31-35 of Gosner (1960). Among their striking characters are the presence of peculiar articular surfaces between cornua trabeculae and suprarostral cartilage, the incomplete development of the orbital cartilage, the high fenestration of the floor of the cavum cranii, the fusion of posterior foramina, and the fusion of spicules 3 and 4 in the hyobranchial skeleton.

Introducción

Diversos autores han tratado la morfología externa de las larvas de Pseudidae. En tiempos recientes Kenny (1969), Duellman & Trueb (1985), Emerson (1988) y Cais & Vizotto (1993) se ocuparon de *Pseudis paradoxa*, haciendo hincapié en el gigantismo corporal, en tanto que Dixon et al. (1995) analizaron los cambios ontogenéticos en el patrón de coloración. Fernandez & Fernandez (1921) y De Sá & Lavilla (1997) caracterizaron la larva de *Pseudis minuta*, y Kehr & Basso (1990) hicieron lo propio con la de *Lysapsus mantidactylus*.

Si bien contamos con información adecuada sobre la morfología externa, es muy poco lo que sabemos sobre otros aspectos larvales. Por ejemplo, la información relativa a caracteres anatómicos es escasa y antigua, y está restringida a las descripciones del condrocráneo (Parker, 1882) y esqueleto visceral (Parker, 1882; Ridewood, 1898) de *Pseudis paradoxa*.

Sabemos que la familia Pseudidae es uno de los agrupamientos enigmáticos en el conjunto de anuros neotropicales. Sus relaciones filogenéticas no han sido satisfactoriamente dilucidadas, dado que no se han identificado apomorfías que la separen claramente de Hylidae, su aparente grupo hermano (Duellman & Trueb, 1985, Ford & Cannatella, 1993; Hay et al., 1995), y la estructura interna de la familia también es materia de discusión. aunque sólo se han reconocido dos géneros, su composición específica necesita revisión.

Tomando en cuenta lo que se acaba de decir, y considerando que la suma de caracteres larvales puede ayudar a solucionar algunos de los problemas enunciados, es el objectivo de

este trabajo describir el condrocráneo y esqueleto visceral de larvas de *Pseudis minuta*, un taxon cuya atribución genérica fuera materia de disputa hasta no hace mucho tiempo.

Material y métodos

La descripción del condrocráneo y esqueleto visceral está basada en cinco larvas en estadios comparables a 31-35 de Gosner (1960) (una por estadio), que forman parte de un lote mayor depositado en la colección herpetológica del Museo Nacional de Historia Natural, Smithsonian Institution, bajo los números USNM 497619 a 497639. El material fue obtenido en laboratorio a partir de puestas naturalmente inducidas de una pareja proveniente de Laguna del Cisne, Salinas, Departamento Canelones, Uruguay (27 X.94, A. Olmos y R. De Sá col.). La hembra está depositada como ejemplar de referencia como USNM 498369. Los renacuajos fueron criados en acuarios de 40 litros con una densidad de 25 ejemplares por acuario para estandarizar variables dependientes de la densidad, y alimentados ad libitum con comida para peces carnívoros.

El material estudiado fue fijado en formol 10 % y teñido diferencialmente para hueso y cartílago, y posteriormente diafanizado, según la técnica de Dingerkus & Uhler (1977). Las observaciones se realizaron bajo glicerina en una lupa binocular Wild M3C

Resultados

Neurocráneo (fig. 1a-c)

El *cartílago suprarrostral* es una estructura única, fuerte y completamente condrificada, que se dirige hacia adelante y hacia abajo a partir del extremo distal de los cuernos trabeculares. El cuerpo presenta una profunda escotadura dorsal en forma de V, y se une a las alas por medio de una banda de cartílago relativamente ancha, que deja una escotadura ventral a cada lado, de márgenes irregulares. Las alas son cuadrangulares; el proceso dorsal posterior es proporcionalmente delgado y con extremo romo, y está proyectado hacia afuera y hacia atrás. El proceso ventral posterior no está definido. En el margen proximal de cada ala, próximo a su unión con el cuerpo, existe un área engrosada que actúa como superficie articular con el cuerno trabecular respectivo.

Los *cuernos trabeculares* corresponden aproximadamente al 17 % de la longitud del neurocráneo. Son estructuras fuertes, completa y uniformemente condrificadas y divergen hacia adelante. El extremo distal está levemente expandido, y los márgenes interno y anterior son irregulares. Ventralmente, en el ángulo externo de cada cuerno se observa un área cartilaginosa engrosada, que se corresponde con la superficie articular descripta para el suprarrostral. Próximo a la región basal de cada cuerno, sobre su margen externo, se insinúa el proceso lateral, la lámina cartilaginosa y el proceso prenasal están ausentes.

Los cuernos trabeculares se continúan hacia atrás con la porción trabecular del piso del neurocráneo, y en esa región no se han diferenciado aún estructuras tales como la placa etmoidal, el septo nasal, el techo nasal ni la lámina orbitonasal.

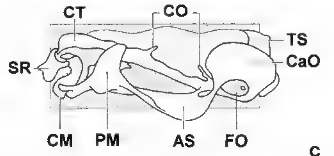


Fig. 1 Condrocráneo de *Pseudis minuta*, estadio 33 de GOSNER (1960) (a) Vista dorsal. (b) Vista ventral. (c) Vista lateral. AS, arco subocular, C, cuadrado, CaO, cápsulas óticas; CCCA, comisura cuadrado-craneal anterior; CM, cartílago de Meckel, CO, cartílago orbital, COc, cóndilos occipitales, CT, cuerno trabecular; FBC, fenestra basicraneal, FCP, foramen carotídeo primario; FO, fenestra oval + operculum; FY, foramen yugular, IR, cartílago infrarrostral, N, notocorda, PA, proceso ascendente, PM, proceso muscular; SR, cartílago suprarrostral, TS, techo sinótico.

Los *cartílagos orbitales* son vestigiales, estando limitados a un par de proyecciones cartilaginosas estrechas, oblicuas, una anterior y otra posterior, y que serían homólogas a las pilas metoptica y antotica respectivamente. El espacio comprendido entre estas dos estructuras está abierto y no se reconocen foramenes de manera individual. La proyección posterior del cartílago orbital no tiene contacto con la cápsula ótica, de modo que el foramen proótico está abierto dorsalmente. No existen tenia tecti marginalis ni tenia tecti transversa, y en la parte media del techo sinótico se observa una proyección triangular hacia adelante, que correspondería a un esbozo de tenia tecti medialis.

El *piso de la cavidad craneal* está poco condrificado y muestra la fenestra basicraneal abierta y proporcionalmente muy grande, correspondiendo al 40 % de la longitud del neurocráneo. Los forámenes craneopalatinos estarían incluidos en dicha fenestra, mientras que los forámenes carotideos primarios son circulares, pequeños y están claramente definidos. En la región posterior, el arco occipital está bien desarrollado y fusionado a las cápsulas óticas, los cóndilos occipitales están esbozados pero aún no osificados, los foramenes yugulares están definidos y la notocorda penetra por un distancia equivalente al 25 % de la longitud del piso de la cavidad craneal.

Las *cápsulas óticas* son cuadrangulares, oblongas y corresponden a aproximadamente el 35 % de la longitud del neurocráneo. La fenestra oval es grande (equivale al 1/3 de la longitud de la cápsula ótica) y el operculum, diferenciado como un elemento cartilaginoso, subcircular y pequeño, ocupa aproximadamente 1/6 de la abertura. La cresta parótica no se reconoce como una estructura discreta, aunque desde el ángulo anterior externo, e inmediatamente por delante de la fenestra oval, surge el proceso ótico larval (en el sentido de De Beer, 1937), dirigido hacia adelante y hacia abajo, formando un arco. En la región posterior ventral de cada cápsula ótica se observa un sólo foramen, de aproximadamente la mitad del tamaño de la fenestra oval, que correspondería a la fusión de los foramenes acústico, perilinfáticos y endolinfático.

Dorsalmente las capsulas oticas están unidas por el *techo sinótico*, en forma de una banda cartilaginosa que presenta en la región media del margen anterior la proyección subtriangular ya mencionada.

Suspensorio

En el suspensorio, el *proceso ascendente* tiene un desarrollo similar al del proceso ótico y se une al piso del neurocráneo (unión baja).

El *arco subocular* se presenta como una lámina delgada, claramente ensanchada en los tercios medio y posterior y curvada hacia abajo.

En el *cuadrado*, el proceso muscular es subtriangular, de margenes irregulares y extremo romo. Está fuertemente curvado hacia adentro, de modo que su porción distal se presenta casi paralela al cuadrado. El margen posterior del proceso coincide con el margen posterior de la comisura cuadrado craneal anterior. Esta comisura, con áreas de condrificación débil, lleva en su margen anterior el proceso cuadrado etmoidal, y en el posterior el proceso pseudopterigoideo. Ambos procesos tienen desarrollo similar, son subtriangulares y de vertice agudo. La fosa hiocuadrado es poco notable, y el proceso articular, condilar, se muestra como un

engrosamiento cartilaginoso subtriangular y romo, ubicado en el margen lateral externo del cuadrado, a nivel de la base del proceso muscular. El túnel muscular es abierto, y está limitado por abajo por la base del cuadrado y la comisura cuadrado-craneal anterior, y por su margen externo y dorsalmente por el proceso muscular.

MANDÍBULA INFERIOR

Los *cartílagos de Meckel* son subcilíndricos y contorneados, con el proceso retroarticular protruido y romo, más un pequeño proceso, también romo, ubicado en el margen interno, a nivel del ángulo. Se unen a los infrarrostrales por medio de cópulas intermandibulares ligamentosas.

Los *cartílagos infrarrostrales*, pares, son oblongos y curvados, y llevan una proyección posterior por la que articulan con los cartílagos de Meckel La cópula intramandibular es conectiva.

ESQUELETO VISCERAL (FIG. 2a-b)

En el esqueleto hiobranquial no se reconoce la *copula I* Los *ceratohiales* están mejor condrificados distal que proximalmente, muestran el proceso hiocuadrado oblongo, protruido y bien desarrollado y los procesos anterior y lateral subtriangulares y notables.

La *pars reuniens* está muy débilmente condrificada y es de contorno aproximadamente rectangular y más ancha que larga.

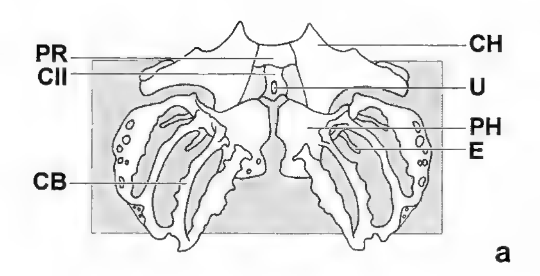
La *copula II*, aunque poco definida, está mejor desarrollada que la estructura anterior; es aproximadamente dos veces más larga que ancha, con el extremo distal angular, y lleva un *proceso urobranquial* corto y romo.

La copula II está relacionada con las *placas hipobranquiales*, muy poco condrificadas, por tejido conectivo.

Los *ceratobranquiales I a IV* constituyen las estructuras mejor desarrolladas del esqueleto hiobranquial y distalmente están unidos entre si por comisuras terminales, mientras que la unión con las placas hipobranquiales se realiza a través de bandas de tejido escasamente condrificadas. Los ceratobranquiales II y III, por su parte, se unen entre sí por medio de un proceso branquial fuerte. Ventralmente existen dos espículas delgadas y poco condrificadas (que corresponden a los ceratobranquiales I y II), mas una placa irregular, poco condrificada, cribosa y contínua con las placas hipobranquiales, que continúan los ceratobranquiales III y IV (fig. 2b).

DISCUSIÓN Y CONCLUSIONES

La ausencia de información sobre la estructura del condrocraneo en miembros del género *Lysapsus* nos impide, por el momento, señalar el conjunto de caracteres derivados compartidos por los Pseudidae y que podrían emplearse para dilucidar sus relaciones con



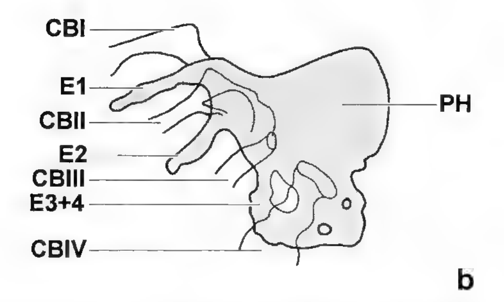


Fig. 2. Esqueleto visceral de *Pseudis minuta*, estadio 33 de Gosner (1960). (a) Vista general. (b) Detalle de placa hipobranquial. CB, ceratobranquiales; CBI, ceratobranquial I, CBII, ceratobranquial II, CBIII, ceratobranquial III, CBIV, ceratobranquial IV, CH, ceratohial, CII, copula II, E, espículas, E1, espicula 1; E2, espicula 2, E3+4, espícula 3 y 4 fusionadas, PH, placa hipobranquial, PR, pars reuniens; U, proceso urobranquial

Hylidae. Hasta obtener dicha información creemos conveniente analizar un conjunto de caracteres del condrocráneo y esqueleto visceral de las larvas de *Pseudis minuta* que llaman la atención por no haber sido reportados previamente entre los anuros conocidos, o por ser comunes a lo reportado para *Pseudis paradoxa* pero muy poco frecuentes en larvas de tipo IV.

(1) Llama la atención los variados patrones de condrificación del esqueleto cefálico. Existen regiones fuertemente condrificadas (i.a., cartílagos supra e infrarrostral, cuernos trabeculares), otras donde sólo se hacen evidentes las paredes de los condrocitos y otras más donde el tejido condrogénico muestra una estructura irregular y difusa (como ciertas regiones del piso del cráneo)

(2) La presencia de dos superficies articulares engrosadas en el margen proximal del cartílago suprarrostral, en la región de unión de cuerpo y ala, es también un carácter particular. Estas estructuras se corresponden con superficies articulares de características similares ubicada en la región ventral del margen anterior de cada cuerno trabecular. Las dos superficies articulares son planas, y se mantienen en posición y se flexionan por medio de ligamentos. La ilustración brindada por PARKER (1882: lám 2 fig 1) muestra una estructura aparentemente similar a la que aquí se describe. Por otra parte, la estructura del cartílago suprarrostral sería derivada, considerando las discusiones de FABREZI & LAVILLA (1992), PLASOTA (1974) y SOKOL (1981).

(3) Los cuernos trabeculares son continuos hacia atrás con la porción trabecular del piso del neurocráneo, sin que se hayan desarrollado aún (estadio 35 de GOSNER, 1960) las estructuras características de la región etmoidal PARKER (1882) reportó la presencia de una estructura equivalente al septo nasal en *Pseudis paradoxa*.

(4) Los cartílagos orbitales, escasamente desarrollados, están representados por un par de pilares, uno en el extremo anterior (asimilado tentativamente a la pila metoptica) y otro en el posterior (¿pila antotica?) del piso del neurocráneo, dejando un gran espacio vacío entre ellos. JACOBSON (1968) y SOKOL (1981) consideraron a la ausencia de cartilago orbital (tal como se observa en algunos Microhylidae) como derivada; la presencia de los pilares extremos mostraría una condición intermedia.

(5) En correlación a la ausencia de un cartílago orbital continuo, el proceso ascendente se une directamente al piso del neurocráneo, una condición considerada como altamente derivada por FABREZI & LAVILLA (1992), y que los asemeja a algunos hílidos (i.a., *Phyllomedusa sauvagii, P boliviana, Phasmahyla guttata, Hyla nana, Scinax acuminatus*).

(6) La gran fenestración del condrocraneo larval de *Pseudis minuta* se acentúa al considerar el notable desarrollo de la fenestra basicraneal, equivalente, como dijéramos, al 40 % de la longitud total del craneo. La mencionada fenestra se obtura en estadios tempranos de desarrollo en *Pseudis paradoxa*, tal como se desprende de la descripción de PARKER (1882). Es conveniente resaltar que los órganos del sistema nervioso central están rodeados por una fascia conectiva muy resistente y firmemente adherida a los elementos esqueléticos de la región.

(7) En la región posterior del cráneo se destaca la fusión de los foramenes acústico, perilinfáticos y endolinfático. El foramen resultante, de gran tamaño, se ubica en la region posterior ventral de cada cápsula ótica.

(8) El palatocuadrado muestra al menos dos caracteres notables. Uno es la expansión proporcionalmente grande de las regiones media y posterior del arco subocular, y otro es el notable desarrollo del proceso ótico larval, que alcanza proporciones similares a la del proceso ascendente. Ambos muestran condiciones equivalentes en *Pseudis paradoxa*.

(9) La posición del proceso muscular del palatocuadrado, ubicado de modo que forma parte del techo del túnel muscular, es un estado de carácter derivado, si se tiene en cuenta el análisis de Fabrezi & Lavilla (1992).

(10) *Pseudis minuta* y *P. paradoxa* comparten la presencia de los procesos cuadrado etmoidal y pseudopterigoideo en los márgenes anterior y posterior de la comisura cuadrado-craneal anterior, respectivamente. En *P. paradoxa* el proceso cuadrado-etmoidal de cada lado está en contacto con el proceso lateral de la base del cuerno trabecular, limitando completamente a la coana, en *P. minuta* los procesos laterales están poco desarrollados y la coana está abierta anteriormente.

(11) La estructura de las espículas en el esqueleto hiobranquial es también peculiar. Los ceratobranquiales I y II están seguidos por espículas de estructura clásica, en tanto que los ceratobranquiales III y IV se continúan en una placa cuadrangular, poco condrificada y cribada, formada por la fusión de las espículas 3 y 4 (fig. 2b). Dichas placas son continuas con la placa hipobranquial respectiva. Una condición similar fue reportada por Parker (1882) y Ridewood (1898) para *Pseudis paradoxa*.

Resumen

Se describe el condrocráneo y esqueleto visceral de las larvas de *Pseudis minuta* en base a 5 ejemplares en estadios 31 a 35 de Gosner (1960). Entre los caracteres peculiares observados se encuentran la presencia de una superficie articular particular entre los cuernos trabeculares y el cartílago suprarrostral, el desarrollo incompleto del cartílago orbital, la gran fenestración del piso del cráneo, la fusión de los foramenes posteriores de la región ótica y la fusión de la espículas 3 y 4 en el esqueleto hiobranquial.

Agradecimientos

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The adult skeleton of *Spea multiplicata* and a comparison of the osteology of the pelobatid frogs (Anura, Pelobatidae)

Anne M. MAGLIA

Division of Herpetology, Natural History Museum and Biodiversity Research Center, and Department of Ecology and Evolutionary Biology, The University of Kansas, Lawrence, Kansas 66045-2454, USA
E-mail: magliaa@ukans.edu

Among the pelobatids (Anura, Pelobatidae), the skeletal anatomy of the North American genera *Spea* and *Scaphiopus* is poorly known. Based on dry-skeletal and cleared and double-stained specimens, I describe the osteology of *Spea multiplicata* and compare it to that of all other pelobatid taxa (*Spea*, *Scaphiopus*, *Pelobates*). Several anatomical structures are shared by *Spea* and *Scaphiopus*, including the absence of a quadratojugal bone, the presence of a palatine process of the facial process of the maxilla, a long postchoanal process of the vomer, and a completely cartilaginous sternum. *Spea* is characterized by a poorly developed maxillary process of the nasal, the lack of a well-developed posteromedial process of the parasphenoid, and possibly a well-developed pars ascendens plectri of the auditory apparatus. Most other diagnostic features of *Spea* relate to the limited cranial ossification of this genus relative to other members of the family.

INTRODUCTION

Among "basal" frogs, the largest and arguably the most poorly known group is the Pelobatoidea. These frogs comprise about 95 extant species (FROST, 1985) in three families (Pelobatidae, Megophryidae and Pelodytidae), and are distributed throughout the Holarctic Region extending into the Old World tropics (DUELLMAN & TRUEB, 1994). Among the Pelobatidae are frogs in the genera *Pelobates*, *Scaphiopus* and *Spea*. Although the skeletal anatomy of frogs in the genus *Pelobates* has been considered by several authors (e.g., ANDERSEN, 1978, ROČEK, 1981; RODRÍGUEZ TALAVERA, 1990), the adult osteology of the North American genera *Spea* and *Scaphiopus* remain poorly understood.

Of the few authors who have considered the skeleton of the North American pelobatids, JURGENS (1971) included *Spea intermontana* in his description of the nasal cartilages of anurans, RAMASWAMI (1939) described the cranial osteology of *Scaphiopus holbrookii*, and FABREZI (1992) described the carpus of *Scaphiopus couchii*. The only thorough description of the anatomy of these frogs is that by WIENS (1989) on the osteological development of *Spea bombifrons*. It is in part because of the lack of detailed morphological descriptions of *Spea*

and *Scaphiopus* that the phylogenetic relationships within the family Pelobatidae are unresolved (FORD & CANNATELLA, 1993) Therefore, I provide a detailed description of the adult skeleton of *Spea multiplicata*, a species for which the anatomy is relatively unknown, and compare its skeleton to that of other frogs in the family Pelobatidae, with the hope of attaining information that may be phylogenetically useful.

MATERIALS AND METHODS

Osteological descriptions of *Spea multiplicata* were made from male and female dried skeletons and cleared and double-stained specimens. Dry-skeletal and cleared and double-stained specimens of *Spea bombifrons*, *S. hammondii*, *S. intermontana*, *Scaphiopus couchii*, *S. holbrookii*, *S. hurterii*, *Pelobates cultripes*, *P. fuscus*, *P. syriacus* and *P. varaldii* also were examined (app. 1). Osteological terminology is that of DE SÁ & TRUEB (1991), TRUEB (1993), DUELLMAN & TRUEB (1994) and FABREZI & ALBERCH (1996, for manus and pes). Descriptions and illustrations were made with the aid of a stereo microscope equipped with a camera lucida.

RESULTS

CRANIUM

The cranium is square and well ossified, but lacks dermal ornamentation (fig 1) Both the neopalatine and quadratojugal are absent in this species. The frontoparietal fontanelle is exposed as a moderate-sized fenestra, and the maxillae and premaxillae bear teeth

Nasal cartilages

The septum nasi is extensively ossified, synostotically fused to the sphenethmoid, and extends forward anterior to the nasal roofing bones. The tectum nasi also is ossified and is invested by the medial margins of the nasals. The oblique cartilages, which form the anterodorsal roof of the nasal capsule, are confluent anteromedially with the septum and tectum nasi and posterolaterally with the commissura lateralis (fig 2). A minute and blunt anterior maxillary process projects forward from the anteroventral border of the planum antorbitale toward the posterior half of the facial process of the maxilla. The posterior maxillary process projects posteriorly from the posteroventral margin of the planum antorbitale, and is fused synchondrotically to the pterygoid process of the palatoquadrate cartilage. The anterolateral margin of the oblique cartilage unites with the robust crista subnasalis, which extends ventrally to abut the anterior margin of the facial process of the maxilla. Posteriorly, the crista subnasalis fuses with the solum nasi, the horizontal sheet of cartilage extending medially from the septum nasi that forms the floor of the nasal capsule. A small, bifurcate process extends posteriorly from the solum nasi to articulate with the sphenethmoid and the dorsal surface of the vomer. The cup-shaped alary cartilage lies above the anterior margin of the solum nasi, providing support for the anterior margin of the nares. The alary cartilage is united synchon-

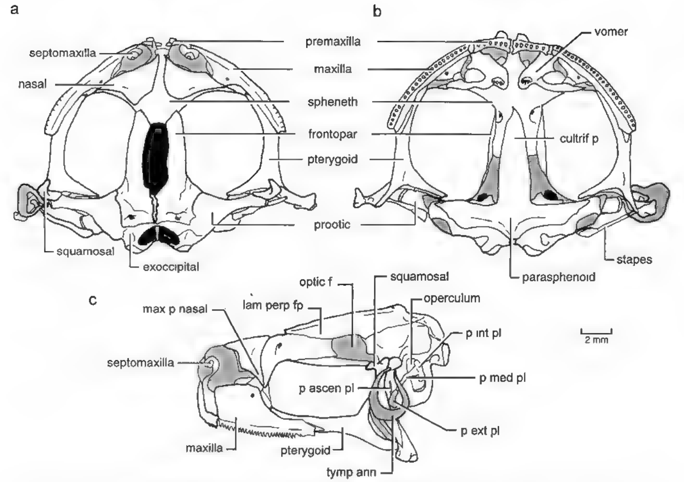


Fig. 1 Cranium of *Spea multiplicata* (KU 86662) in (a) dorsal, (b) ventral, and (c) lateral view. Gray denotes cartilage, black denotes foramina. Abbreviations: cultrif p, cultriform process of parasphenoid; f, foramen; frontopar, frontoparietal; lam perp fp, lamina perpendicularis of frontoparietal; max p nasal, maxillary process of nasal; p acen pl, pars acendens plectri; p ext pl, pars externa plectri; p int pl, pars interna plectri; p med pl, pars media plectri; spheneth, sphenethmoid; tymp ann, tympanic annulus.

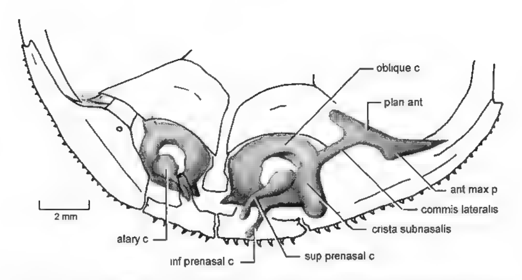


Fig. 2 Nasal cartilages of *Spea multiplicata* (KU 86664) in oblique anterior view Gray denotes cartilage, white denotes bone. Abbreviations. ant max p, anterior maxillary process; c, cartilage, commis, commissura, inf, inferior; plan ant, planum antorbitale; sup, superior

drotically with the superior prenasal cartilage, which extends ventromedially to the alary process of the premaxilla The inferior prenasal cartilage extends anteroventrally from the solum to the premaxilla.

Septomaxillae

Each of these small bones lies medial to the fusion of the oblique cartilages and the crista subnasalis, and posterior to the alary cartilage (fig. 2). Although complex in shape, only the medial and lateral rami are exposed in dorsal view (appearing to be U-shaped).

Sphenethmoids

These extensively ossified elements are fused dorso- and ventromedially to form a single bone, exposed dorsally in a diamond-shaped area between the frontoparietals and the nasals (fig. 1a). Anteriorly, the ossified septum nasi is synostotically fused to the sphenethmoid, forms the shared medial wall of the nasal capsules, and extends forward beyond the length of the nasal roofing bones. Ventrally, the sphenethmoid floors the nasal capsules, extending anteriorly to the level of the pars palatina of the premaxilla (fig 1b) The sphenethmoid extends laterally for most of the planum antorbitale to articulate with the pars facialis of the maxilla. Ventromedially, the orbitonasal foramen opens posteriorly at the level of the anterior margin of the cultriform process. Dorsomedial ossification includes the anterior margin of the frontoparietal fontanelle; posteroventral and lateral ossification continues to the level of the anterior margin of the optic foramen, thereby forming the anterolateral wall of the neurocranium.

Prootics and exoccipitals

The prootics and exoccipitals are synostotically united with one another. The prootics form the anterior and ventrolateral parts of the otic capsule, and are invested dorsomedially by the frontoparietals (fig. 1a). The posterolateral margin of the frontoparietal extends to the eminentia epiotica. Each prootic forms the posterior margin of the optic foramen; anterior ossification extends only to the posterior three fourths of the prootic foramen. Laterally, the prootic narrows to form an extensively ossified crista parotica, with only the distal tip being cartilaginous. The lateralmost tip of the prootic, ventral to the crista parotica, is mineralized and articulates with the basal process (sensu Reiss, 1997) of the pterygoid.

The exoccipitals form the posteromedial part of the otic capsule, as well as the margin of the foramen magnum and the occipital condyles. The hyal of the hyoid attaches to the posterolateral margin of the exoccipital, and possibly to the posterior margin of the basal process, via a small ligament (or other unstained connective tissue). The margin of the foramen magnum is incompletely ossified dorsomedially and dorsoventrally (fig 1b). The occipital condyles, lateral to the foramen magnum, are well developed. Internal and slightly lateral to the occipital condyles are the jugular foramina.

Plectral apparatus

The plectral apparatus is ventral to the crista parotica, oriented horizontally (fig. 1b-c). The fully ossified pars interna plectri is expanded but separate from the fenestra ovalis and operculum. The operculum is robust and completely ossified, except for the posterolateral margin. The pars media plectri is columnar, slightly sigmoidal, and expanded medially to articulate with the pars interna plectri. Distally, the pars externa plectri forms a flat cartilaginous plate that fills about one-third of the tympanic annulus. A well-developed pars ascendens plectri extends from the medial portion of the pars externa plectri to the crista parotica. The tympanic annulus attaches dorsally to the cartilaginous tip of the crista parotica, and except for a slight separation at this articulation, forms a complete ring

Nasals

The rhomboidal, paired nasals overlie the nasal capsule (fig. 1a). Medially, they overlap the septum nasi of the sphenethmoid, although this element is clearly visible between them. Posteriorly, the nasals overlap the planum antorbitale, but do not articulate with the frontoparietals. Laterally, the poorly-developed maxillary process of each nasal narrows to extend to the level of the pars facialis of the maxilla, but does not articulate with it.

Frontoparietals

These paired, dorsal elements form the lateral and posterior margins of the frontoparietal fenestra (fig 1a) Anteriorly, they invest the sphenethmoid to the level of the tectum anterior; anterolaterally, each narrows away from the anterior margin of the fenestra and lacks a supraorbital flange. Laterally, each forms the lamina perpendicularis, which extends

ventrally about one third of the height of the braincase and posteriorly to the anterior margin of the optic foramen (fig. 1c). Posterodorsally, these elements overlap the prootic to the eminentia epiotica. A narrow ridge, the occipital crest, forms anterior to the eminentia epiotica. Anterior to this crest, the occipital foramen opens posteriorly. Although completely covered, the occipital canal is visible through the bone, traversing obliquely from the lateral margin of the frontoparietal to open at the level of the posterior margin of the frontoparietal fenestra. In some specimens, a smaller foramen opens dorsally at the midpoint of the occipital canal.

Parasphenoid

The parasphenoid is broad, smooth, and lacks bony ornamentation. The anterior half of the broad cultriform process overlaps the sphenethmoid, and narrows to a point just posterior to the level of the planum antorbitale (fig. 1b). The parasphenoid alae are broad, anterolaterally oriented, and ventrally invest the otic capsule. A distinct posteromedial process is absent; however, the posterior margins of the alae converge to form the posteromedial margin. This part underlies the ventral cartilaginous margin of the foramen magnum (between the exoccipitals).

Vomers

The vomers are large, bear about five teeth each, and contribute to the floor of the nasal capsules (fig. 1b). The anterior process is rectangular and extends obliquely from its anterior margin just posterior to the maxilla-premaxilla articulation toward the midline of the body. At the level of the dentigerous process, a small prechoanal process extends laterally. Medial to this process is a small foramen for the palatine ramus of the facial nerve. The dentigerous process is rounded, and narrowly separated from its counterpart. The postchoanal process is long and slender, and invests the planum antorbitale. This process extends beyond the planum antorbitale to articulate at its most dorsolateral end with the anterior ramus of the pterygoid via the posterior maxillary process of the planum antorbitale and may articulate weakly with the pars facialis of the maxilla.

Premaxillae

The premaxillae are narrowly separated from one another; each has a well-developed alary process that is inclined anteriorly, curved slightly laterally, and ends dorsally in a bifurcated, rounded tip. The pars dentalis curves dorsolaterally; its anteroventral surface appears wavy because of the presence of approximately 12 teeth (fig. 1b). The palatine process of the pars palatina is a short, flat plate that forms a right triangle. A small posterolateral process of the pars palatina also is present.

Maxillae

Each maxilla possesses approximately 36 teeth and lacks pre- and postorbital processes. The pars facialis of each is well developed and reaches its maximum height at the level of

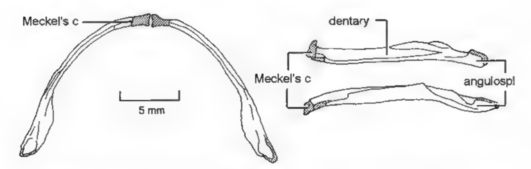


Fig 3. Mandible of *Spea multiplicata* (KU 86664) in dorsal (left), lateral (right, top), and lingual (right, bottom) view. Gray denotes cartilage. Abbreviations: angulospl, angulosplenial; c, cartilage.

the planum antorbitale (fig. 1c). The anterior tip of the pars facialis articulates with the lateral margin of the premaxilla and the medial margin articulates with the planum antorbitale of the sphenethmoid. A small foramen, possibly a foramen for a ramus of the maxillaris superior vessels, traverses vertically through the pars facialis.

Mandible

The angulosplenial, dentary, and mentomeckelian bones comprise the mandible (fig. 3). The mentomeckelian bones form the anterior margin of the mandibles; they are small and relatively well ossified, and are fused to one another medially. The thin dentary articulates with the posterior portion of the mentomeckelian, and extends posteriorly for more than half the length of the mandible, investing the lateral margin of Meckel's cartilage. The angulosplenial forms the posterior portion of the mandible and serves as the attachment point for the mandible to the cranium. The angulosplenial extends anteriorly to invest most of the lingual margin of Meckel's cartilage. Posteromedially, the angulosplenial possesses a well-developed coronoid process.

Squamosals

The zygomatic ramus of the squamosal is short and projects anteriorly (fig. 1c). The otic ramus of the squamosal invests the anterolateral tip of the crista parotica. The ventral ramus extends posteroventrally at a 45° angle relative to the horizontal axis of the skull and invests the ossified portion of the palatoquadrate cartilage. A thin, sheetlike process extends anteromedially from the ventral ramus, ventral to the zygomatic ramus, and invests the palatoquadrate cartilage

Pterygoids

The triradiate pterygoids are well developed, with robust anterior and medial rami (fig. 1a-b). The anterior ramus projects anterodorsally, invests the pterygoid process of the palatoquadrate, and articulates with the pars palatina of the maxilla. The anterior ramus

synchondrotically fuses to the lateral margin of the postchoanal process of the vomers. The posterior ramus invests the ventrolateral surface of the pars articularis of the palatoquadrate. The medial ramus invests the pterygoid process of the palatoquadrate and articulates with the basal process.

Palatoquadrates

The pars articularis of the palatoquadrate (quadrate process) is ossified to the level of midheight of the ventral process of the squamosal. The basal process extends medially to articulate with the prootic, and is invested by the medial ramus of the pterygoid (fig. 1c).

HYOID APPARATUS

Hyoid apparatus

There is little sexual dimorphism in the hyoid apparatus. The hyoid plate shows no mineralization and is narrow, the length along the longitudinal axis (midlength) is about two-thirds the length along the transverse axis (fig. 4). The hyoglossal sinus is U-shaped. Separate anterolateral processes are not present; they are fused to the hyoid plate in development, creating oval lateral foramina (WIENS, 1989), which are larger in males. As in other pelobatoids (CANNATELLA, 1985), the hyals are disassociated from the hyoid plate, with each ventrally investing the lateral margin of the hyoid plate, posterior to the lateral foramen, narrowing posterolaterally, and extending forward to articulate with the exoccipital (or basal process of the palatoquadrate; see *Exoccipitals* above).

The slender posterolateral processes project from the posterior margin of the hyoid plate at approximately a 45° angle to the transverse axis of the hyoid plate. These processes are about equal in length to the midlength of the hyoid plate. The ossified posteromedial processes project posterolaterally from the posteromedial margin of the hyoid plate at approximately a 60° angle to the transverse axis of the hyoid plate. In males, the shaft of each posteromedial process is one-third the width of the proximal and distal heads; in females, the shaft is half the width of either head.

Laryngeal cartilages

There is sexual dimorphism in both the size and shape of the laryngeal cartilages. In males, the laryngeal apparatus nearly fills the entire space between the posteromedial processes; in females, only half of this space is filled. In ventral view, the paired arytenoid cartilages, which are much larger in males, lie within the cricoid ring. As each of these cartilages extends dorsomedially, it becomes more narrow and less concave, and appears to form discrete dorsal and ventral parts (fig. 4). In males, the dorsal portion extends almost the full length of the ventral portion. In females, the dorsal portion is only half the length of the ventral part, and the anterodorsal margin is acuminate. The elongate, paired bronchial processes project ventrolaterally from the cricoid ring at the level of the distal heads of the posteromedial processes. The distal portion of each bronchial process terminates in a head with three

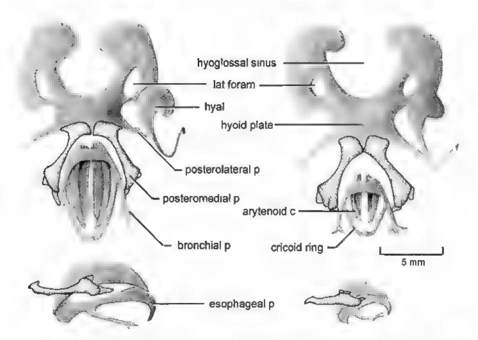


Fig 4. – Ventral view of hyoid apparatus (top) and lateral view of cricoid ring (bottom) of male (left, KU 86664) and female (right; KU 86662) *Spea multiplicata*. Stippled pattern denotes bone, gray denotes cartilage. Abbreviations: c, cartilage; lat foram, lateral foramen; p, process.

fingerlike projections. In males, the bronchial processes extend to the level of the posterior margin of the arytenoid cartilages; in females, these processes extend to the level of the posterior margin of the cricoid ring. Slightly posterior to the origin of the bronchial processes, shelf-like expansions extend medially from the cricoid ring. In males, a small square esophageal process extends ventrally from the posterior margin of the cricoid ring; in females, this process is less distinct.

Axial skeleton

The vertebral column is composed of eight notochordal presacral vertebrae, the sacrum and the urostyle (fig. 5a). The vertebrae are slightly imbricate, and ossified intervertebral bodies are present between the centra. Each neural arch bears a low neural ridge with two small, posterior projecting spinous processes; the articular facets of the pre- and postzygapophyses are simple. The relative lengths of transverse processes and sacral diapophyses are as follows: III > sacrum ≈ IV > II > V ≈ VI ≈ VII ≈ VIII. Transverse processes of presacrals III-V are almost perpendicular to the notochordal axis, whereas those of presacrals II, VI, VII and VIII are directed anteriorly. Small, posteriorly directed uncinate processes are present on the transverse processes of vertebrae II-IV.

The cervical cotyles of the atlas are Type II (Lynch, 1973) and are nearly contiguous. The urostyle is rounded in cross section, fuses with the sacrum, and bears a dorsal ridge

Fig. 5 (a) Dorsal view of axial skeleton of *Spea multiplicata* (KU 86664). (b) Ventral view of pectoral girdle (KU 86662), with the scapula and suprascapula deflected ventrally. (c) Ventral (left) and lateral view of pelvic girdle (KU 86664) Gray denotes cartilage. Abbreviations. c, cartilage, f, fossa; procor, procoracoid; sacral diap, sacral diapophysis, suprascap, suprascapula.

throughout its anterior two thirds. The sacrum consists of vertebrae IX and X, and the slightly expanded sacral diapophyses (expanded transverse processes of vertebrae IX, Wiens, 1989) are oriented perpendicular to the midline of the body. A bony webbing, which has been mistaken for postsacral transverse processes (discussed by Wiens, 1989), is present on the posterior margin of the sacrum, between vertebrae IX and X.

Appendicular skeleton

Pectoral girdle

The sternum is a spade-shaped plate of cartilage that floats between the epicoracoid cartilages, typical of the arciferal arrangement of the girdle (fig. 5b). A completely cartilaginous, knob-shaped omosternum articulates with the epicoracoid bridge of the epicoracoid cartilages. The anterior margins of the paired procoracoid cartilages are completely invested by the clavicles and are synchondrotically contiguous posteromedially with the epicoracoid cartilages. The pectoral fenestrae are large and tear-shaped, each is bordered anteriorly by the procoracoid cartilage, medially by the epicoracoid cartilage, posteriorly by the coracoid, and laterally by the glenoid fossa.

The relatively long clavicles (one-third longer than the coracoids) are posteriorly concave; the glenoidal end of each is flared anteriorly, forming a wedge-shaped process that abuts the pars acromialis of the scapula. The clavicles do not reach the midline and are separated medially by the epicoracoid bridge. The long axes of the coracoids are slightly arcuate; each of these robust bones is narrowly separated anterolaterally from the clavicle and articulates with the pars glenoidalis of the scapula. The sternal end of each coracoid is moderately broad (twice the width of the shaft), but narrower than the glenoidal end (approximately 80 % of width of glenoidal end). The scapular end of the coracoid is also broad (almost three times the width of the shaft) and its distal concavity articulates with the pars acromialis, forming the posterior surface of the relatively deep glenoid fossa.

The scapula is about three times the length of the glenoid fossa, with its greatest width being half of its total length. The pars glenoidalis is a thin, concave plate, and the pars acromialis is a robust, rounded process; both form the remaining portion of the glenoid fossa. The shaft of the scapula is short and constricted (width about one-fourth total length of scapula). The distal head of the scapula is expanded to articulate with the cleithrum; its width is twice the width of the shaft and half the total length of the scapula. The cleithrum invests most of the anterior two-thirds of the suprascapular cartilage. It is narrow anterodistally and broadens at the scapular end to form the shape of a cleaver. The suprascapular cartilage extends posteriorly as a broad, flat blade.

Forelimb

The humerus has a large, flangelike crista ventralis, a slightly smaller, well-developed crista medialis, and a low crista lateralis. The glenoid head of the humerus (caput humeri) is cartilaginous, whereas the distal head (eminentia capitata) is completely ossified. The flattened radioulna is about two-thirds the length of the humerus and its distal head is wider than

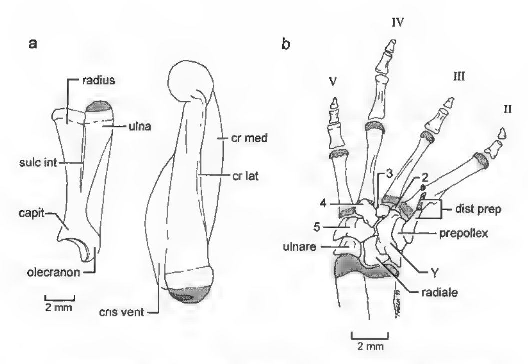


Fig. 6. - Right forelimb of *Spea multiplicata* (KU 86664); top of figure is anterior. (a) Dorsal view of radioulna (left) and lateral view of humerus. (b) Ventral view of hand. Gray denotes cartilage. Abbreviations. capit, capitulum, cr lat, crista lateralis; cr med, crista medialis, cris vent, crista ventralis; dist prep, distal prepollex; sulc int, sulcus intermedius, u, ulnare, Y, element Y.

its proximal head (fig. 6a). A distinct groove, the sulcus intermedius, distinguishes the radius and ulna, although they are fused to one another medially. A small flange is present along the proximolateral edge of the ulna.

The manus resembles that of *Scaphiopus couchii* (morphology A) as described by FABREZI (1992). Proximally, there is a small ulnare, and a slightly larger radiale; distally, there is a large irregular-shaped element Y and a smaller carpal 5 (fig. 6b). Carpals 2, 3 and 4 lie proximal to metacarpals II, III and IV, respectively. Carpals 3 and 4 are partially fused to one another, and lie on the ventral surface of the manus; carpal 2 is smaller, and lies dorsal to element Y. All carpal elements are well ossified. A moderate-sized, ossified prepollex lies distomedial to element Y. One completely ossified distal prepollical element and at least one cartilaginous distal prepollex also are present. Relative lengths of the digits are IV > II > III ≈ V. The phalangeal formula is 2-2-3-3. There is apparent sexual dimorphism in the size and shape of digit II, in male specimens, the metacarpal and phalanges are thickened, with a small protuberance on the medial border of the metacarpal.

Pelvic girdle

In dorsal view, the internal margins of the ilia form a narrow U-shape (fig. 5c). The ilial shafts are simple, with no obvious crests, but have a small dorsal prominence. The preacetabulum is moderate and the preacetabular angle (i.e., the angle between the ilial shaft and the preacetabular margin) is slightly obtuse. The ilia are separated from one another medially and from the ischia posteriorly by cartilage. The ischia are approximately D-shaped, and are fused to one another to form the posterior margin of the acetabulum. The completely cartilaginous pubis forms the ventral border of the acetabulum.

Hind limb

The femur is long and thin (length about 12 times width); both the distal and acetabular heads are cartilaginous. The femur possesses a small ridge on the lateral margin. The tibiofibula is about three-fourths the length of the femur, and its distal and proximal heads are of similar size. Although the tibia and fibula are fused, a distinct groove separates them. The tibiale and fibulare are short and robust (length less than half that of the tibiofibula), and are fused to one another at their proximal and distal heads (fig. 7a).

The pes has a single ossified tarsal element proximal to digit II, and a large element Y (FABREZI, 1993) proximal to metatarsal I (fig. 7b). An ossified prehallux and a large spadelike distal prehallical element are present medial to element Y. Relative lengths of the digits are IV > V > III > II > I. The phalangeal formula is 2-2-3-3-3.

DISCUSSION

Although the anatomy of frogs in the genus *Pelobates* is relatively well known, the phylogenetic relationships within the family Pelobatidae are unresolved (FORD & CANNATELLA, 1993), in part because of a lack of detailed morphological descriptions of the other members of the family, *Spea* and *Scaphiopus*. The description provided herein should facilitate a more detailed comparison among pelobatid taxa. What follows is both a summary of the most recent works on pelobatid osteology as well as my own observations. The preliminary comparisons of *Spea multiplicata* to all other pelobatid taxa presented here were incorporated as part of a phylogenetic analysis of the pelobatids (MAGLIA, 1998).

Most recent authors (e.g., FORD & CANNATELLA, 1993, DUELLMAN & TRUEB, 1994) agree that pelobatids (*Pelobates*, *Scaphiopus* and *Spea*) form a monophyletic assemblage. However, although there are several diagnostic characters for these frogs (including broad sacral diapophyses and sculpturing of dermal cranial bones, ROČEK, 1981), few osteological features have been proposed to be shared derived characters uniting *Pelobates*, *Scaphiopus* and *Spea*. CANNATELLA (1985) proposed that the presence of cranial exostosis and a long zygomatic ramus of the squamosal were synapomorphies for the pelobatids, however, both of these features are absent in the genus *Spea*. He also cited the presence of a supraorbital flange of the frontoparietal in all pelobatids, however, I have not seen evidence of this structure in any

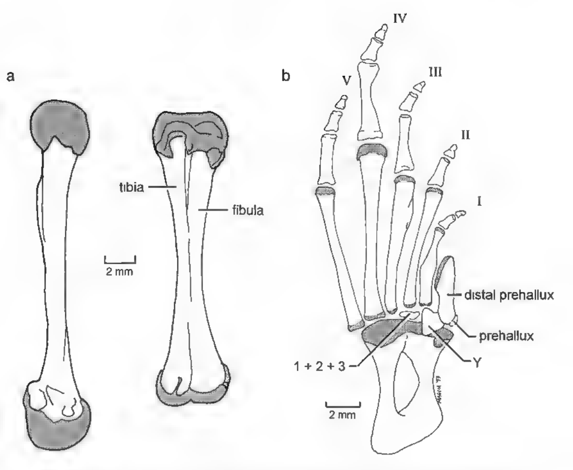


Fig. 7. Right hind limb of *Spea multiplicata* (KU 86664) (a) Lateral view of femur (left) and ventral view of tibiofibula (b) Ventral view of right foot Gray denotes cartilage Abbreviation Y, element Y

Spea The other osteological characters uniting the pelobatids in CANNATELLA's (1985) analysis, the complete ossification of septum nasi and the fused articulation of the urostyle and sacrum, are found in several non-pelobatid taxa.

In comparing the osteology of *Spea multiplicata* with that for all other pelobatid taxa, I found several features shared by the pelobatids. All of these taxa possess an occipital canal that is roofed completely by bone. This feature is not present in non-pelobatid pelobatoids (e.g., *Megophrys*, *Pelodytes*), however, it is present in other taxa (e.g., some neobatrachians LYNCH, 1969, MENDELSON et al., in press). The presence of bony webbing on the posterior margin of the sacral diapophyses is shared among all pelobatids. This has been identified by some authors (e.g., LYNCH, 1973, DUELLMAN & TRUEB, 1994) as post-sacral transverse processes, but was shown to originate in development from the sacral diapophyses (WIENS,

1989). Other morphologies shared by all pelobatids are the presence of relatively elongate, convex clavicles and well-developed facial and preorbital processes of the maxilla.

The North American pelobatids *Spea* and *Scaphiopus* have several morphological features that are unique to them, including the lack of a quadratojugal bone and the presence of a palatine process of the facial process of the maxilla (CANNATELLA, 1985) These taxa also possess a postchoanal process of the vomer that subtends the planum antorbitale (discussed in CANNATELLA, 1985) and a completely cartilaginous sternum. ROČEK (1981: 151) provided a detailed comparison of the cranial differences between *Pelobates* and the North American pelobatids, and included a discussion of several features common to *Scaphiopus* and *Spea* (e.g., well-developed stapes, ossified operculum).

Several morphologies are unique to the genus *Spea*. For example, *Spea* lacks the exostosis of the dermal cranial and suspensorium elements found in all other pelobatids. Also, the otic ramus of the squamosal barely overlaps the crista parotica, whereas it forms an otic plate investing nearly half the otic capsule in other pelobatids. Also in *Spea*, the frontoparietals do not come into contact with the nasals; they lack supraorbital flanges, and they are in contact posteromedially only, exposing the frontoparietal fontanelle. These features most likely relate to the degree of ossification of the cranium of *Spea*: these frogs are much less ossified than other pelobatids. If *Scaphiopus* and *Spea* share a most common ancestor, which seems to be of little doubt (FORD & CANNATELLA, 1993; DUELLMAN & TRUEB, 1994), and if the clade [*Spea* + *Scaphiopus*] is the sister group to *Pelobates* (also well supported; CANNATELLA, 1985), then the limited ossification and small body size of *Spea* may be a reversal of the hyperossification present in *Pelobates* and *Scaphiopus*. However, it is just as likely that the common ancestor shared by the Pelobatidae resembled *Spea* in amount of ossification, and that the hyperossification present in *Pelobates* and *Scaphiopus* evolved separately in these taxa.

Morphologies are thought to be highly conserved among species of *Spea*, and primarily one morphological feature, the frontoparietal boss, has been the subject of much discussion (WIENS & TITUS, 1991) Therefore, the only major works attempting to analyze the relationships within the genus *Spea* have relied on biochemical data (e.g., SAGE et al., 1982, WIENS & TITUS, 1991) However, comparing *S. multiplicata* to other members of the genus, I found several features that vary to some degree among these frogs. For example, the maxillary process of the nasal is poorly developed in *S. multiplicata* and *S. bombifrons*, but is more extensive in the other taxa. Similarly, *S. multiplicata* and *S. bombifrons* lack a well-developed posteromedial process of the parasphenoid, whereas the other taxa possess this feature.

A small but striking anatomical feature present in *Spea multiplicata* is a well-developed pars ascendens plectri of the auditory apparatus. Because this feature can only be observed on cleared and double-stained specimens with well-developed plectral apparatuses, I was able only to compare it among a few taxa in this sample (*S. bombifrons*, *Scaphiopus couchii* and *Pelobates varaldii*). Of these, the pars ascendens plectri was only present in *S. bombifrons* (although not described by WIENS, 1989) This structure may be unique to *Spea* or may vary among pelobatids. It is hoped that further comparisons of this feature and others discussed here will help in resolving the relationships among the pelobatids.

RESUMEN

Entre los pelobátidos (Anura, Pelobatidae), la morfología esqueletal de los géneros norteamericanos *Spea* y *Scaphiopus* es pobremente conocida. La osteología de *Spea multiplicata* se describe en base a esqueletos secos y a especímenes diafanizados y doblemente teñidos, y se la compara con todos los otros taxones de pelobátidos (*Spea*, *Scaphiopus*, *Pelobates*). *Spea* y *Scaphiopus* comparten varias morfologías, incluyendo la ausencia de cuadradojugal, la presencia de un proceso palatino del proceso facial de la maxila, proceso postcoanal del vomer largo, y esternon completamente cartilaginoso. *Spea* se caracteriza por un proceso maxilar del nasal pobremente desarrollado, falta de un proceso posteromedial de parasfenoides bien desarrollado y posiblemente una pars ascendens plectri del aparato auditivo bien desarrollada. La mayoría del resto de los caraceres diagnósticos de *Spea* están relacionados a la limitada osificación craneal de este género en relación a otros miembros de la familia.

ACKNOWLEDGEMENTS

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APPENDIX 1
SPECIMENS EXAMINED

INSTITUTIONS

AMNH: American Museum of Natural History, New York, USA.
KU: The University of Kansas, Lawrence, USA.
MCZ· Harvard University Museum of Comparative Zoology, Cambridge, USA.
MNCN: Museo Nacional de Ciencias Naturales, Madrid, Spain.

SPECIMENS EXAMINED

Pelobates cultripes: KU 148619, MNCN 20041
Pelobates fuscus: KU 68819, 129240
Pelobates syriacus: KU 146856.
Pelobates varaldii· AMNH 62935, MCZ 31970
Scaphiopus couchii· KU 20444, 73384, 209575
Scaphiopus holbrookii· KU 20439, 145413.
Scaphiopus hurterii: KU 20472, 60173, 90096.
Spea bombifrons: KU 5405, 73382
Spea hammondii: KU 176016.
Spea intermontana: KU 79436, 204563
Spea multiplicata KU 27622, 39776A B, 49468, 84888, 86662, 86664, 97355, 106225, 117347

Corresponding editor Masafumi MATSUI

Geographic variation of *Hyla rubicundula* and *Hyla anataliasiasi*, with the description of a new species (Anura, Hylidae)

Marcelo Felgueiras Napoli & Ulisses Caramaschi

Departamento de Vertebrados, Museu Nacional do Rio de Janeiro,
Quinta da Boa Vista, 20940-040, Rio de Janeiro, RJ, Brasil
E-mail: napoli@acd ufrj br

Analyses of intra- and interpopulation variation of the external morphology of *Hyla rubicundula* Reinhardt & Lütken, 1862 and *Hyla anataliasiasi* Bokermann, 1972 indicate that four morphospecies are represented. *Hyla rubicundula* comprises three of the four morphospecies. Its northern morphospecies is described as a new species characterized by an immaculate dorsum and a pointed snout. Redescriptions of *H. rubicundula* and *H. anataliasiasi* are provided.

Introduction

The species currently included in the *Hyla rubicundula* group share the following characteristics: small size (SVL: males 16.0-25.5 mm, females 16.6-25.9 mm), thighs immaculate, dorsum consistently green in life, and dorsal surfaces pink to violet in preservative. This group occurs in northern, central, northeastern and southeastern Brazil (Frost, 1985), in open habitats, mainly in "cerrado" formations, but also in transitional areas between cerrado and rainforests.

According to Bokermann (1968) and Frost (1985), the *Hyla rubicundula* group is composed of *Hyla rubicundula* Reinhardt & Lutken, 1862, *Hyla tritaeniata* Bokermann, 1965 and *Hyla anataliasiasi* Bokermann, 1972. *Hyla elongata* A. Lutz, 1925 was synonymized with *H. rubicundula* by Bokermann (1968) but treated as a valid species by Haddad et al. (1988); the latter authors compared vocalizations of specimens from Serra da Canastra, Minas Gerais, with the vocalizations of topotypic populations of *H. rubicundula* described by Cardoso & Vielliard (1985), and considered *H. rubicundula* and *H. elongata* as distinct species. However, our examination of the external morphology of the specimens from Serra da Canastra revealed that they must be associated to the *H. tritaeniata* complex, and were wrongly identified as *H. elongata* by Haddad et al. (1988). Thus, the synonymization of *H. elongata* with *H. rubicundula* proposed by Bokermann (1968) is valid.

Hyla tritaeniata, originally included in the *H. rubicundula* group, is not treated in this paper because it has (1) a distinctive dorsal pattern (a single sacral stripe, instead of two in the other species) and (2) different habitat preferences: this species is found in springs and streams, whereas the rest of the group inhabits permanent or temporary ponds (Bokermann, 1965, Jim, 1980). Also, (3) the large intra- and interpopulation variations of *H tritaeniata* suggest a species complex that must be analyzed separately.

The purposes of this paper are (1) to study the degree of intra- and interpopulation variation in *H. rubicundula* and *H anataliasiasi*, and (2) to describe a new species of the *H rubicundula* species group.

Material and methods

Specimens used for description or examined for comparisons were previously deposited in the collections of the Museu Nacional, Rio de Janeiro (MNRJ), of the Museu de Zoologia, Universidade de São Paulo (MZUSP), of the Naturhistorisches Museums, Vienna (NMW), of the Werner C A. Bokermann collection, deposited in the Museu de Zoologia, Universidade de São Paulo, SP, Brazil (WCAB), of the Kobenhavns Universitet, Zoologisk Museum, Copenhagen (ZMUC), and of the Museu de História Natural, Universidade Estadual de Campinas (ZUEC). The analysis of the material was similar to that used by Vanzolini (1970) and Heyer (1984). Initially, large samples from each locality were analyzed ("basic samples") to determine the patterns of variation within samples. Specimens were sorted into morphospecies (i e., categories thought to represent different species). Subsequently, samples from poorly represented localities were analyzed ("small samples"), and these specimens, when possible, were associated to a morphospecies by similar morphology and proximity among localities. The last step of the analysis corresponds to a careful examination of the patterns of variation among morphospecies.

Only adult males were examined because females and juveniles were rare in the samples. We developed a series of standards for the general dorsal pattern, mid-dorsal pin stripe, dorsolateral stripes, lateral limits of dorsum, upper surface of tibia, loreal and canthal stripes, and dorsal head shape (fig. 1-3). Nine measurements (mm) were taken following Duellman (1970): SVL (snout-vent length), HL (head length), HW (head width), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), IND (internarial distance), TD (tympanum diameter) and TL (tibia length) Four measurements were made following Heyer et al. (1990): UAR (upper arm), FAR (forearm), HAL (hand length) and THL (thigh length) Five other measurements were END (eye to nostril distance: straight line distance between anterior corner of orbital opening and posterior margin of external nare), NSD (nostril to tip of snout distance: straight line distance between anterior corner of nostril to tip of snout), FL (foot length distance from heel to tip of fourth toe), 3FD (third finger disk diameter. greatest horizontal distance between outer edges of third finger disk) and 4TD (fourth toe disk diameter greatest horizontal distance between outer edges of fourth toe disk). Webbing formula notations followed Savage & Heyer (1967).

Discriminant function analyses compared inter- and intra-morphospecies variation (Marcus, 1990) without removing the size effect in the groups (Reis et al., 1990), and groups

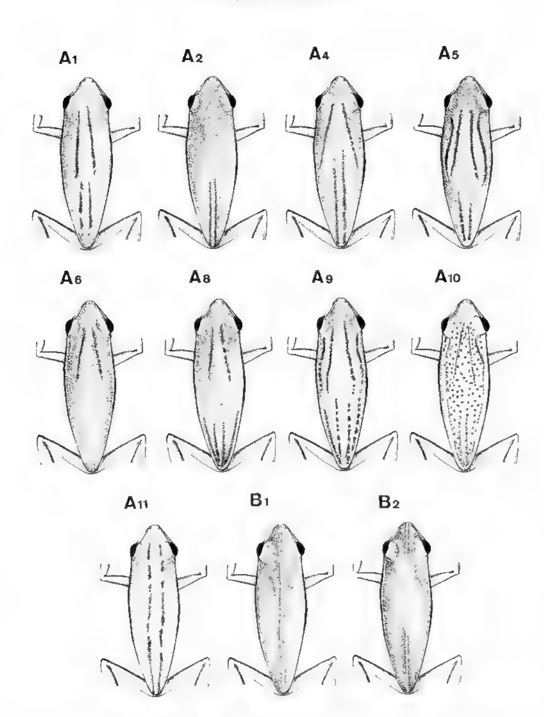


Fig. 1. Standards for dorsal and mid-dorsal pin stripe patterns. Patterns A3 (dorsum immaculate), A7 (one to few dots distributed irregularly) and B3 (absence of mid-dorsal pin stripe) are not figured

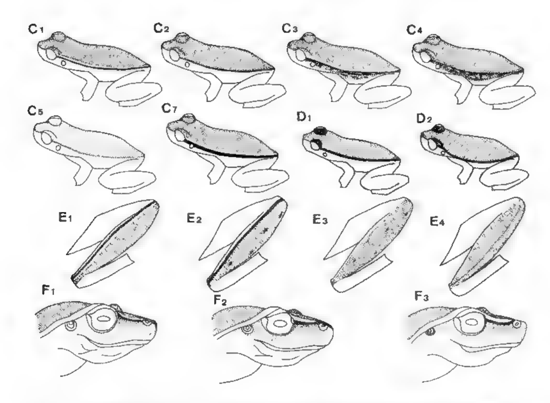


Fig. 2 - Standards for dorsolateral stripes: C1-C2, thin and regular; C3-C4, thick and irregular; C5, vestigial; C6, absent, is not figured, C7, thick and well marked. Lateral limits of dorsum: D1, above the tympanum; D2, under the lower border of tympanum. Upper surface of tibia patterns. E1, white stripe over dark stripe; E2, white stripe absent, E3, white and dark stripes vestigial or absent, E4, presence of a mid-dorsal pin stripe. Loreal and canthal stripes patterns F1, thin white stripe over dark stripe; F2-F3, thick clear band over dark stripe.

were defined a priori. Eigenvectors and associated eigenvalues were obtained from a variance-covariance matrix, and the loadings were the correlations between the original variables and the scores. We used *t*-tests to compare mean values from different measurement variables of the same species. For character analyses, we used the chi-square test (χ^2) to compare patterns among samples of the same morphospecies (Sokal & Rohlf, 1981).

Vocalizations were recorded by Rogério P. Bastos with a Uher Report Monitor and a Uher M 518 A microphone at a tape speed of 19 cm/s. Tapes were analyzed on a Macintosh Classic coupled to a MacRecord Sound System 2.0.5

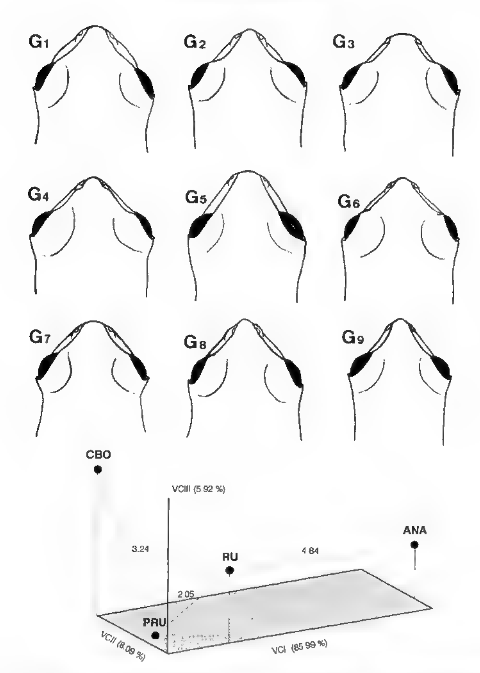


Fig. 3. Standards for the dorsal head shape patterns (G1-G7), and projection of centroids resulted from the multiple discriminant function analysis for 18 morphometric characters of the combined samples of morphospecies RU, PRU, CBO and ANA, in the first three canonical axes. A minimum spanning tree connects the closest means, and the Mahalanobis distance is given for each link of the tree, this procedure corrects the distortion caused by the three-dimensional projection

Results and discussion

Morphospecies

The four morphospecies were named and coded as follows (code, code name, number of specimens analyzed, localities):

RU, *Hyla rubicundula*, $n = 144$. Bahia: Barreiras and Jupaguá. Minas Gerais: Alfenas, Andrequicé, Arinos, Barão de Cocais, Belo Horizonte, Buritis, Buritizeiro, Esmeraldas, Jaboticatubas, Januária, Lagoa Formosa, Lagoa Santa, Manga, Pirapora, Três Marias, Unai and Vespasiano. Goiás: Cristalina.

PRU, *Hyla* "pseudorubicundula", $n = 54$ Minas Gerais: Uberlândia. Goiás. Aragarças, Cavalcante, Goiânia, Iaciara, Monte Alegre de Goiás, Nova Roma, Porangatu, Santa Rita do Araguaia, São Domingos and escarpa da Serra Dourada. Piauí: Uruçui.

CBO, "Cachimbo", $n = 15$. Pará: Cachimbo.

ANA, *Hyla anataliasiasi*, $n = 85$. Mato Grosso Posto Leonardo and Posto Diauarum.

Comparisons among morphospecies

Results from the analysis of the seven coloration patterns indicate two categories of characters (tab. 1). In the first category, frequencies of character states differed among morphospecies, but no states (e.g., mid-dorsal pin stripe or loreal and canthal stripes patterns) were diagnostic. The second category was defined by states unique to certain morphospecies, and specimens having such unique states were easily diagnosed from the other morphospecies (e.g., any specimen that presented pattern A11 for general dorsal pattern was automatically assigned to morphospecies ANA). General dorsal patterns, dorsolateral stripes, lateral limits of dorsum, upper surface of tibia, and dorsal head shape patterns belonged to this category. Taken in combination, pattern characteristics distinguished most but not all individuals of the four morphospecies; that is, a specimen that had only character states common to all morphospecies was not assigned to one of them.

Measurement variables

Multiple discriminant function analysis was used to analyze morphological variation among the four morphospecies. We found three significant axes (Wilks $\lambda = 0.0753$, $F = 16.86$, $df = 54$ and 659.3, Bonferroni corrected, $P < 0.01$) (fig. 3). Morphospecies ANA and CBO were easily discriminated from morphospecies RU and PRU, but the last two were only partially discriminated from each other (tab. 2). The standardized discriminant function coefficients and the loadings are presented in tab. 3.

Table 1. - Distributions and percentage (in parentheses) of patterns (fig. 1-3) among the four morphospecies. A blank indicates no specimen had that state; a zero indicates that at least one specimen with that state was examined, but the rate of occurrence per 100 specimens rounds off to zero. *n* = number of specimens for which data are available.

General dorsal patterns												
Morphospecies	*n*	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
RU	132	53 (40)	1 (7)	15 (11)	17 (12)	4 (3)	6 (4)	2 (1)	9 (6)	15 (11)	1 (0)	
PRU	48	16 (33)	4 (8)	1 (25)	2 (4)	2 (4)	1 (2)	1 (2)	2 (4)	7 (14)	1 (2)	
CBO	15			5 (33)				11 (66)				
ANA	81		1 (1)	45 (55)			11 (13)	5 (6)				19 (23)

Mid-dorsal pin stripe					Dorsolateral stripes							
Morphospecies	*n*	B1	B2	B3	*n*	C1	C2	C3	C4	C5	C6	C7
RU	144	64 (43)	32 (21)	48 (33)	132	83 (62)	49 (37)					
PRU	48	2 (4)	10 (20)	36 (75)	46	5 (10)	8 (17)	15 (32)	10 (21)	3 (6)	5 (10)	
CBO	15			15 (100)	15		14 (93)	1 (6)				
ANA	82	36 (43)	20 (22)	26 (31)	81	15 (18)	50 (61)			10 (12)	1 (1)	5 (6)

Lateral limits of dorsum				Upper surface of tibia					Loreal and canthal stripes				
Morphospecies	*n*	D1	D2	*n*	E1	E2	E3	E4	*n*	F1	F2	F3	F4
RU	134	134 (100)		134	91 (67)	37 (26)	6 (3)		140	119 (84)	12 (7)	6 (4)	3 (2)
PRU	48	22 (42)	26 (54)	45	12 (26)	27 (60)	6 (13)		46	4 (8)	22 (47)	14 (30)	6 (13)
CBO	15	15 (100)		15		10 (66)	5 (33)		15		9 (60)	6 (40)	
ANA	82	82 (100)		82	3 (3)		13 (15)	66 (80)	82	40 (46)	4 (4)		38 (46)

Dorsal head shape										
Morphospecies	*n*	G1	G2	G3	G4	G5	G6	G7	G8	G9
RU	140	9 (6)	40 (28)	58 (41)	9 (6)	11 (7)	11 (7)	2 (1)		
PRU	47		5 (10)	2 (4)	1 (2)	1 (2)		38 (80)		
CBO	15								15 (100)	
ANA	82									82 (100)

Variation within morphospecies RU

The analysis examined the samples from Minas Gerais and Bahia. These samples were grouped into four areas equidistantly distributed along a transect (fig. 4A) linking Barreiras (Bahia) and Alfenas (Minas Gerais) that represented, respectively, the distribution limits north and south for morphospecies RU. Distributions of pattern states were determined for each of the four areas, and the observed occurrences were tested against expected occurrences (based on frequency of distribution for entire sample RU) with a chi-square test. Some character states were combined to avoid violating minimum cell-size requirements for χ^2 analysis (app. 1; Sokal & Rohlf, 1981).

Three directional clines were observed (fig. 4A). The first direction (shading "A") denoted a cline for general dorsal pattern and upper surface of tibia pattern (fig. 5A). These specimens showed an increase in dorsal melanization and a decrease of the dorsolateral white stripe on the edges of tibia from southeastern to northeastern Minas Gerais. The second direction (shading "B") denoted a cline for dorsal head shape (fig. 5A) involving areas I, II and IV. We did not consider area III because it is not representative (the two geographical samples in the direction "B" included only two specimens and neither were well preserved), thus, there is a hiatus between areas II and IV. The third cline followed the transect line. It was characterized by a decrease in occurrence of a mid-dorsal pin stripe (fig. 5A) from south to north (i.e., from area I/II to IV). The patterns of loreal and canthal stripes and dorsolateral stripes did not show statistically significant level variation.

The similarity among these areas depended on each particular character, and there was no specific pattern discriminating an area from the others. However, differentiation may be computed in the degree of occurrence for a certain state. The similarity and dissimilarity among areas shown by each character obtained from the χ^2 test was as follows: general dorsal pattern (I ≈ IV; II ≈ III), mid-dorsal pin stripe pattern (I ≈ II; III ≈ IV), dorsolateral stripes pattern (I ≈ II ≈ III; IV), upper surface of tibia pattern (I ≈ II ≈ IV; III), loreal and canthal stripes pattern (I ≈ IV ≈ II ≈ III), and dorsal head shape (I ≈ III; II ≈ IV).

Measurement variables

Multiple discriminant function analysis was used to analyze morphological variation among nine samples previously combined. To increase the number of specimens analyzed, samples from Três Marias and Andrequicé, Pirapora and Lagoa Formosa, and Vespasiano and Barão de Cocais were combined because of their proximity. Three significant canonical axes (Wilks $\lambda = 0.02385$, $F = 3.274$, $df = 144$ and 712.6; Bonferroni corrected, $P < 0.0006$) resulting from this analysis represented 79 % of the total variation. The projection of the individual scores in the first three axes (not figured) did not support additional discrimination and made a mosaic of superpositions among the geographic samples. This result may be interpreted as intraspecific variation. All samples were considered to belong to *H. rubicundula*.

VARIATION WITHIN MORPHOSPECIES PRU

This analysis examined samples from Goiás. These were grouped into three areas (fig. 4B) with the same criteria as for morphospecies RU, but the small number of specimens in each sample, mainly in areas I and III, made the use of the χ^2 test (pattern analysis) impossible in most comparisons. The discriminant function analysis used to analyze morphological variation (measurement variables) among five previously combined samples furnished only one significant canonical vector (Bonferroni corrected) without any relevant discrimination result.

Frogs from areas I and II were similar to each other in the majority of characters but were different from those from area III. A cline, characterized by the straight line between Santa Rita do Araguaia and São Domingos (fig. 4B), was observed for (1) dorsolateral stripes (a progressive disappearance of the dorsolateral white stripe from northern to southern Goiás) and (2) dorsal head shape patterns (a decrease of diversity of dorsal head shape patterns from northern to southern Goiás; fig. 5B) The similarity among areas shown for each character, obtained for certain characters by the χ^2 test, is as follows: general dorsal pattern (I ≈ II; III), mid-dorsal pin stripe pattern (I ≈ II; III), dorsolateral stripes pattern (I ≈ II; III), lateral limits of dorsum pattern (I ≈ II; III), upper surface of tibia pattern (I ≈ II; III), loreal and canthal stripes pattern (I, II, III) and dorsal head shape (I ≈ II; III). Differences between areas I and II were mainly by degree of occurrence of some states, rather than kind; area III differed from the others by degree and kind.

TAXONOMIC CONCLUSIONS

Morphospecies RU and PRU were not well discriminated from each other. Pattern standards denoted variation in degree between these morphospecies but not in kind. Such variation occurred for all character similarity between area III of Minas Gerais (fig. 4A) and area I of Goias (fig. 4B) The discrimination obtained by the discriminant function analysis was not robust (tab. 2) Also, the comparisons between advertisement calls of topotypic *Hyla rubicundula* (CARDOSO & VIELLIARD, 1985) (morphospecies RU) and a sample from Silvânia, Goiás (morphospecies PRU, see *Vocalization* in *Hyla rubicundula* redescription below) failed to provide additional support for discrimination.

The distribution of morphospecies PRU in Goiás (central Brazil) deserves consideration The Serra do Caiapó, Serra Dourada, Serra dos Pirineus and heterogeneous vegetation separate the examined population samples in three areas in northern, southern and eastern Goiás (Goiânia). The vegetation (ANONYMOUS, 1989) is mainly represented by seasonal semi-deciduous forest, seasonal deciduous forest and transitional areas ("ecological stress areas") Because these frogs never cross tropical rainforests, the discontinuity of cerrado formation in central Brazil, where different kinds of relief and vegetation are found, may reduce or obstruct genetic flow among local populations and favor the formation of heterogeneous morphotypes.

The "Espigão Mestre" (scarps, 1200-3000 m), with tropical rainforests, between Goias and Bahia, as well as the semi-deciduous seasonal forest of southern Goias (ANONYMOUS, 1989) adjacent to Minas Gerais, may function as ecological barriers between populations of

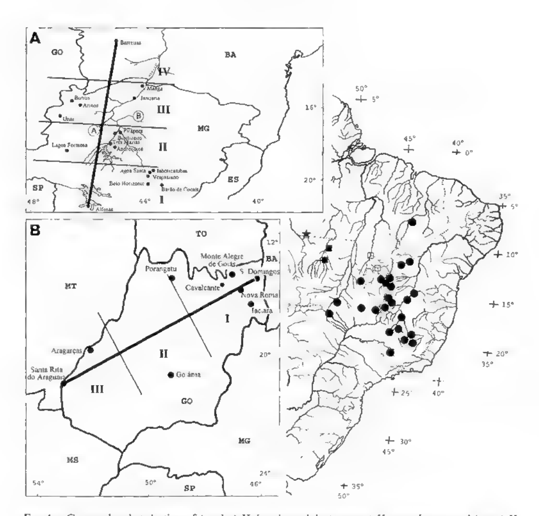


Fig. 4. Geographic distribution of (circles) *Hyla rubicundula*, (squares) *H. anataliasiasi* and (stars) *H. cachimbo*. Each plot may represent more than one sample. Closed symbols show the localities of examined samples, and open symbols the localities of samples of *H. anataliasiasi* not examined in this paper. (A) Distribution of morphospecies RU in Minas Gerais and Bahia. A transect line links Barreiras and Alfenas, the distribution limits north and south for RU. Shading areas A and B show directions of morphological variation explained in text (see *Variation within morphospecies RU*). (B) Distribution of morphospecies PRU in Goias. A transect line links São Domingos and Santa Rita do Araguaia, the distribution limits north and south for PRU. For detailed explanation of each character involved, see *Variation within morphospecies PRU*. BA, Bahia; ES, Espirito Santo; GO, Goias; MG, Minas Gerais; MS, Mato Grosso do Sul; MT, Mato Grosso; SP, São Paulo; TO, Tocantins. Roman numerals indicate areas equidistantly distributed throughout the transect.

RU and PRU which occur only in cerrado habitats. The greatest morphological similarity between these two morphospecies occurs right in the cerrado corridors that allow interactions between populations of RU in Minas Gerais and Bahia and PRU in Goias. We conclude that both morphospecies RU and PRU belong to *Hyla rubicundula*.

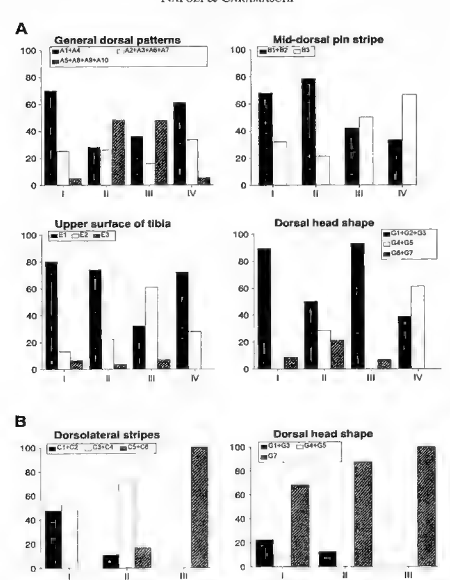


Fig. 5 Frequency (in percentage) of patterns obtained in morphospecies (A) RU and (B) PRU for areas I-IV (fig. 4A) and areas I-III (fig. 4B) respectively. Patterns were combined (for criteria, see app. 1) in order not to violate minimum cell-size requirements for chi-square analysis.

Morphospecies ANA (*Hyla anataliasiasi*) and CBO are well discriminated from each other and from the other two morphospecies (*Hyla rubicundula*) by the analyses of external morphology and morphometrics. Morphospecies CBO is restricted to an isolated savanna which is separated from cerrado by 200 km of tropical rainforest and was probably connected to the cerrado during periods of drier climate (Pleistocene; PRANCE, 1996). As we stated, these frogs never cross tropical rainforests, thus, this geographic isolation obstructs genetic flow and

Table 2. - Classification table for specimens based on the results of the discriminant function analysis for the combined samples RU, PRU, CBO, and ANA; Results presented graphically in fig. 5. *n* = number of specimens.

Morphospecies	*n*	RU	PRU	CBO	ANA
RU	124	96 (77.42%)	23 (18.55%)	4 (3 23%)	1 (0.81%)
PRU	41	5 (12 20%)	33 (80.49%)	3 (7.32%)	0
CBO	12	0	0	12 (100%)	0
ANA	65	0	0	0	65 (100%)

suggests a speciation mechanism. Morphospecies CBO and ANA may be considered full species, and we assigned the following morphospecies to these species: morphospecies RU and PRU to *Hyla rubicundula* Reinhardt & Lütken, 1862, morphospecies ANA to *Hyla anataliasiasi* Bokermann, 1972; and morphospecies CBO to a new species described below.

Species descriptions

Hyla cachimbo sp. nov.

(fig. 6A, 7A, 8A)

Holotype. MZUSP 21912, adult male, collected at Cachimbo (about 09°21'S, 54°57'W), Pará, Brazil, between 200 and 400 m, 18 October - 9 November 1955, by E. Dente, F. S. Pereira and W. Bokermann

Paratopotypes. – Thirteen adult males (MNRJ 17298-17299; MZUSP 21911, 21913-21918, 21920-21926) and an adult female (MZUSP 21910), collected with the holotype.

Diagnosis. – Species characterized by the following combination of traits. (1) small size (SVL: males 19 8-21.0 mm; female 24.2 mm); (2) lateral limits of dorsum above the tympanum (pattern D2, fig. 2); (3) head as long as wide, width contained about 3.1 times in the snout-vent length; and (4) dorsal snout profile acuminate (fig. 6A, 7A)

No specimen of *H. cachimbo* has two divergent dorsal brown stripes from the anterior section of head to near the middle of the body nor two parallel sacral stripes, but many individuals of *H rubicundula* have such a pattern (patterns A1, A2, A4-A6 and A8-10, fig. 1). No specimen of *H cachimbo* has a mid-dorsal pin stripe, but many individuals of *H rubicundula* have such a pattern (fig. 1) No specimen of *H. cachimbo* has the lateral limits of dorsum under the lower border of tympanum (pattern D2; fig. 2), but many individuals of *H rubicundula* from Goiás have such a pattern. No specimen of *H cachimbo* has a light pinkish to white stripe above a brown stripe on the edges of the tibia (pattern E1; fig. 2), but many individuals of *H rubicundula* have such a pattern; also, no specimen of the former has a thin

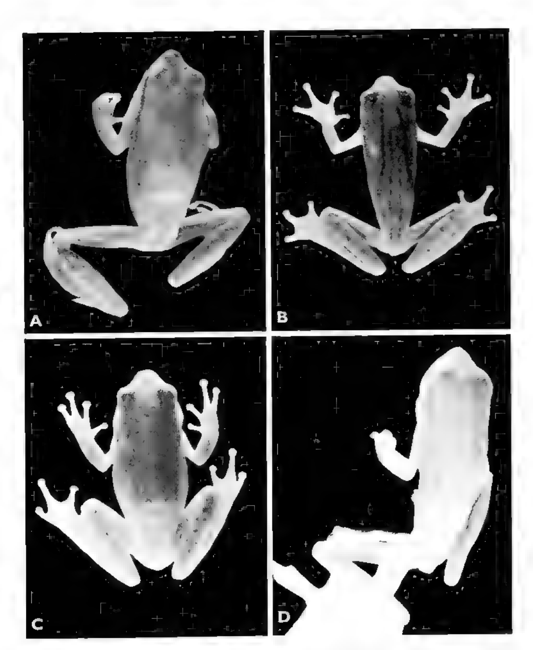


Fig 6 Dorsal views of adult males. (A) *Hyla cachimbo*, holotype, MZUSP 21912, Cachimbo, Para, (B) *H rubicundula*, MNRJ 17294, Lagoa Santa, Minas Gerais, (C) *H rubicundula*, MNRJ 17295, Goiânia, Goiás, (D) *H anataliasiasi*, MZUSP 49610, Posto Diauarum, Mato Grosso.

Table 3. – Standardized discriminant function coefficients for 18 morphometric characters of the combined samples of morphospecies RU, PRU, CBO and ANA. r, correlation coefficient (Pearson) of the original data with the scores resulted from the discriminant function analysis; ^{ns}, not significant; * P < 0.05; ** P < 0.02, *** P < 0.01.

Characters	VC1	VC2	VC3	$r_{(VC1)}$	$r_{(VC2)}$	$r_{(VC3)}$
SVL	0.55	- 0.41	- 0.59	0.73^{***}	-0.24^{***}	0.15^{***}
HW	- 0.85	0.22	- 0.03	0.9^{***}	-0.11^{ns}	0.05^{ns}
HL	0.45	0.12	0.39	0.78^{***}	-0.07^{ns}	-0.04^{ns}
ED	- 0.15	0.43	- 0.65	0.55^{***}	0.16^{***}	0.37^{***}
UEW	- 0.17	- 0.68	0.11	0.69^{***}	-0.45^{***}	0.07^{ns}
IOD	- 0.34	- 0.14	0.31	0.69^{***}	0.01^{ns}	-0.09^{ns}
END	- 0.44	0.33	- 0.05	0.82^{***}	-0.01^{ns}	-0.01^{ns}
IND	- 0.25	- 0.21	0.42	0.81^{***}	-0.08^{ns}	-0.25^{***}
THL	- 0.53	0.81	- 0.68	0.81^{***}	-0.09^{ns}	0.09^{ns}
TL	0.96	- 0.12	1.83	0.7^{***}	-0.16^{***}	-0.04^{ns}
TD	0.16	- 0.18	- 0.23	0.17^{***}	-0.03^{ns}	0.26^{***}
NSD	0.3	0.34	0.08	0.7^{***}	0.04^{ns}	-0.14^{**}
UAR	0.14	0.39	- 0.18	0.58^{***}	0.02^{ns}	0.06^{ns}
FAR	- 0.12	- 0.28	0.19	0.67^{***}	-0.2^{***}	0^{ns}
HAL	- 0.18	0.33	0.07	0.79^{***}	-0.05^{ns}	0.14^{**}
3FD	- 0.37	0.25	0.15	0.85^{***}	-0.07^{ns}	0.01^{ns}
FL	- 0.01	- 1.54	- 0.98	0.79^{***}	-0.28^{***}	0.12^{*}
4TD	0.03	0.15	- 0.24	0.82^{***}	-0.06^{ns}	0.13^{*}

longitudinal central brown stripe composed of small dots, whereas many individuals of *H. anataliasiasi* have such a pattern (pattern E4). The presence in *H. cachimbo* of a broad pinkish stripe above a canthal brown stripe (patterns F2-F3; fig. 2) distinguishes it from *H. anataliasiasi* which presents a canthus well delimited by a thin white stripe above a brown stripe (pattern F1). A pointed snout (fig. 6A, 7A) differentiates *H. cachimbo* from *H. rubicundula* (fig. 6B-C, 7B-C). The head of the former is as long as wide, about 3.1 times into the snout-vent length, and this feature distinguishes it from *H. anataliasiasi* which has a head longer than wide, its width being contained about 3.6 times in the snout-vent length.

Description. – Descriptive statistics are provided in tab. 4. Head as long as wide, its width contained about 3.1 times in snout-vent length; internarial distance greater than eye-nostril distance ($n = 15$, $t = 2.76$, $P = 0.01$) and smaller than eye diameter ($n = 15$, $t = 20.66$, $P = 0$); eye diameter greater than eye-nostril distance ($n = 15$, $t = 19.68$, $P = 0$), snout acuminate in

Table 4. - Descriptive statistical tables of morphometric characters for *Hyla cachimbo* sp. nov. (morphospecies CBO) and *H. anataliasiasi* (morphospecies ANA). *n* = number of specimens for which data are available; *x* = mean; *s* = standard deviation; *CV* = coefficient of variation.

	Morphospecies CBO							Morphospecies ANA										
Characters	Males						Females	Males						Females (*n* = 4)				
	n	*x*	*min*	*max*	*s*	*CV*	(*n* = 1)	*n*	*x*	*min*	*max*	*s*	*CV*	*x*	*min*	*max*	*s*	*CV*
SVL	15	20.74	19.8	21.0	0.64	3.11	24.2	80	18.85	16.0		1.51	8.03	19.70	16.6	21.6	2.24	11.39
HW	15	6.39	6.0	6.8	0.25	3.94	7.7	80	5.28	4.4	21.8	1.40	7.72	5.46	4.6	6.1	0.65	12.01
HL	15	6.49	6.2	6.8	0.21	3.23	7.7	80	5.68	4.7	6.1	0.40	7.05	6.07	5.4	6.8	0.59	9.84
ED	15	2.38	2.2	2.6	0.12	5.23	2.5	80	2.19	1.9	6.5	0.10	4.56	2.27	2.0	2.4	0.15	6.83
UEW	14	1.41	1.2	1.7	0.14	10.12	1.7	77	1.22	0.9	2.4	0.16	13.21	1.15	1.0	1.2	0.09	7.93
IOD	14	2.29	2.0	2.6	0.16	7.36	2.5	78	1.84	1.4	2.2	0.16	9.03	1.95	1.7	2.2	0.22	11.65
END	15	1.54	1.4	1.8	0.10	7.02	1.7	80	1.21	1.0	2.2	0.11	9.66	1.33	1.1	1.6	0.18	14.11
IND	15	1.63	1.5	1.7	0.06	6.95	1.8	80	1.27	1.0	1.6	0.11	8.82	1.32	1.2	1.4	0.09	7.22
THL	15	9.90	9.3	10.5	0.35	3.59	12.5	80	8.38	7.1	1.5	0.69	8.32	8.88	7.3	10.0	1.24	14.01
TL	15	10.01	9.4	10.6	0.33	3.34	12.4	80	8.80	7.5	10.1	0.78	8.86	9.25	7.8	10.5	1.13	12.31
TD	14	1.00	0.8	1.1	0.09	9.6	1.1	73	0.91	0.6	10.6	0.12	13.44	0.87	0.6	1.0	0.16	18.95
NSD	15	1.19	1.0	1.3	0.08	7.14	1.3	80	0.93	0.7	1.4	0.08	9.10	0.92	0.8	1.0	0.08	9.36
UAR	15	6.00	5.7	6.4	0.22	3.71	7.0	80	5.27	4.4	1.1	0.42	8.02	5.35	4.8	5.8	0.45	8.46
FAR	15	3.87	3.6	4.2	0.19	4.95	5.1	80	3.37	2.8	6.3	0.29	8.68	3.43	3.0	3.9	0.39	11.5
HAL	15	5.91	5.5	6.2	0.22	3.72	7.5	80	4.92	4.0	4.0	0.43	8.92	5.13	4.5	5.7	0.54	10.70
3FD	15	0.88	0.7	1.0	0.07	8.10	1.0	80	0.65	0.5	5.9	0.08	12.83	0.71	0.5	0.8	0.11	16.58
FI	15	14.10	13.1	15.1	0.56	3.99	18.9	80	12.31	10.3	0.8	1.12	9.09	13.43	11.0	15.1	1.81	13.53
4TD	15	0.81	0.7	0.9	0.06	8.47	1.0	74	0.59	0.4	14.9	0.08	14.50	0.58	0.5	0.6	0.07	12.76

dorsal outline and protruding or rounded in lateral outline; loreal region slightly oblique; eyes moderately prominent; tympanum distinct and nearly circular; a supratympanic fold being sometimes present, partially covering tympanum; nostrils dorsolateral; internarial region flat; vomerine teeth often present in two patches between choanae; tongue cordiform or ovoid, vocal sac single and subgular.

Forearm more robust and shorter than upper arm ($n = 15$, $t = 28.09$, $P = 0$); hands with a distinct palmar tubercle, subarticular tubercles rounded, distal tubercle of third finger bifid or rounded; distal tubercle of fourth finger always bifid; supernumerary tubercles present, third finger disk diameter greater than fourth toe disk ($n = 15$, $t = 5.72$, $P = 0$); modal webbing formula, I 2 50-2 50 II 2-2.25 III 2.75-2.25 IV. Legs slender; femur and tibia with about the same stoutness and length ($n = 15$, $t = 0.87$, $P = 0.39$); sum of thigh and tibia lengths smaller than snout-vent length ($n = 15$, $t = 3.42$, $P = 0$). Foot with robust toes; subarticular tubercles always rounded, supernumerary tubercles not distinct; prehallux distinct; plantar tubercle distinct; modal webbing formula, I 2^{-}-2.25 II 1.25-2 25 III 1.25-2.75 IV 3^{-}-1.75 V.

Color in preservative. - Dorsum reddish, immaculate, with occasional dark brown dots; mid-dorsal pin stripe absent; canthus rostralis delimited by a subcanthal brown stripe (patterns F2-F3; fig. 2); lorus with variable melanization; a slender lateral brown stripe sometimes present on flanks from posterior corner of orbit to near groin, sometimes bordered by a light pinkish stripe (patterns C2-C3; fig. 2), thigh light brown, immaculate; a brown stripe sometimes present on anterior and posterior edges of upper surface of tibia in addition to dorsal random dots (patterns E2-E3; fig. 2); ventral surfaces immaculate buff Color in life unknown.

Measurements of holotype. - SVL 21.3; HW 6.8, HL 6.8; ED 2.4; UEW 1 4; IOD 2.6; END 1 7; IND 1.5; THL 10.5; TL 10.6, TD 1 0; NSD 1.1; UAR 6.4; FAR 4.2, HAL 6 2, 3FD 0.9; FL 15.1; 4TD 0.8

Etymology. – The specific name, a noun in apposition, refers to the type-locality, Cachimbo.

Geographic distribution. – Known only from the type-locality (fig. 4). This area is characterized as an "ecological stress area" (ANONYMOUS, 1991) or a transitional area between the Cerrado Domain and the Amazon Equatorial Domain (AB' SABER, 1977).

Hyla rubicundula Reinhardt & Lütken, 1862

(fig. 6B-C, 7B-C, 8B-C)

Hyla rubicundula Reinhardt & Lütken, 1862; BOKERMANN, 1968, 1972.

Specimens examined BRAZIL. BAHIA: Barreiras (MNRJ 0934, 0946, 0935-0940, 0933, 6145-6154), Jupaguá (MNRJ 0943-0944). MINAS GERAIS: Alfenas (MNRJ 17126-17128, 17129-17133, 17134); Andrequicé (MNRJ 17110); Arinos (MZUSP 64500-64504), Barão de Cocais (MNRJ 17210-17212), Belo Horizonte (MNRJ 17214-17220, MZUSP 519, 34647); Buritis (MZUSP 64449-64452, 64455-64458, 64460-64464), Buritizeiro (MNRJ 17111-17112, 17113-17116); Esmeraldas (ZUEC 4023); Jaboticatubas (MZUSP 57712-57713), Januária (MNRJ 0942), Lagoa Formosa (MNRJ 17123); Lagoa Santa (topotypes, MNRJ 17117-

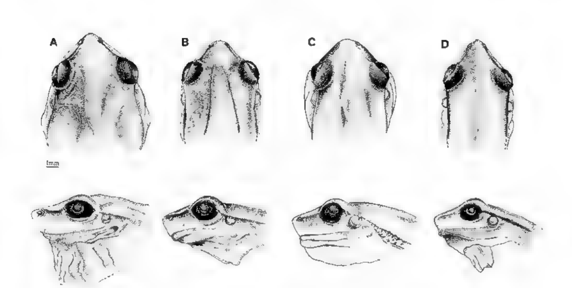


Fig. 7 Dorsal and lateral views of the heads of adult males. (A) *Hyla cachimbo*, holotype, MZUSP 21912, Cachimbo, Para, (B) *H rubicundula*, topotype, morphospecies RU, MNRJ 17294, Lagoa Santa, Minas Gerais; (C) *H rubicundula*, morphospecies PRU, MNRJ 17295, Goiânia, Goias, (D) *H anataliasiasi*, MZUSP 49610, Posto Diauarum, Mato Grosso

17121, 17124-17125, 3081, 13287, 0947, 6155-6177; MZUSP 34012-34023; ZUEC 4150), Manga (MNRJ 0941); Pimenta (MNRJ 17319-17321), Pirapora (MNRJ 0928-0932, 0945, 0923-0927), Santa Luzia (MNRJ 17322-17323); Três Marias (MNRJ 17101-17109), Uberlândia (MNRJ 17305-17308); Unaí (MZUSP 64398-64402, 64386, 64389-64392, 64396; MNRJ 17135), Vespasiano (MNRJ 17221-17223; MZUSP 12691-12693) GOIÁS: Aragarças (MZUSP 20983); Cavalcante (MZUSP 66543, 66570, 66574, 66576), Cristalina (MZUSP 64522), Goiânia (MNRJ 17136-17155, 17300), Iaciara (MZUSP 66527-66528), Monte Alegre de Goiás (MZUSP 66403-66407, 66450, 66456); Nova Roma (MZUSP 66358-66360), Porangatu (MNRJ 17167-17168), Santa Rita do Araguaia (MZUSP 66650-66654), São Domingos (MZUSP 66597-66601, 66602, 66603); escarpa da Serra Dourada (ZUEC 7505) PIAUÍ: Uruçuí (MNRJ 17224).

Syntypes. NMW 16511, ZMUC 1440-1441, Lagoa Santa (about 19°37′S, 43°53′W), Minas Gerais, Brazil, 760 m (BOKERMANN, 1968; FROST, 1985), specimens not examined by us.

Diagnosis. - Species characterized by the following combination of traits: (1) small size (SVL males 18.0-23 4 mm, females 21 6-25 1 mm); (2) in preservative, dorsum with two divergent brown stripes from anterior section of head to sacral region, and two sacral stripes of same color and orientation extending to cloacal region (pattern A1; fig. 1), (3) a thin brown dorsolateral stripe bordered by a thin light stripe from posterior corner of orbit to near groin (pattern C1, fig. 2); and (4) head as long as wide, its width contained about 3 3 times in snout-vent length (fig. 6B-C, 7B-C)

The presence of dorsal brown stripes (patterns A1-A2, A4-A6 and A8-A10; fig. 1) in many individuals of *Hyla rubicundula* differentiate them from *H. cachimbo* which never has such a pattern. The presence in many specimens of the former of two divergent dorsal brown stripes, from the anterior section of the head to nearly the middle of the body, together with two sacral brown stripes (patterns A1 and A4; fig 1), with or without additional brown stripes (patterns A5 and A8-A10), distinguish them from *H. anataliasiasi*, which do not have such patterns. No specimen of *H rubicundula* has the two anterior divergent dorsal brown stripes fused to the sacral ones (pattern A11), whereas many individuals of *H. anataliasiasi* have such a pattern. A mid-dorsal pin stripe (patterns B1-B2; fig. 1) in many specimens of *H. rubicundula* distinguish them from *H. cachimbo*, in which it is often absent. A broad and irregular dorsolateral stripe, with or without an upper white to pinkish stripe (patterns C3-C4, fig. 2) in many specimens of *H. rubicundula* distinguishes them from *H anataliasiasi*, which never has such a pattern The lateral limits of the dorsal coloration in many specimens of *H. rubicundula* are under the lower border of the tympanum (pattern D2; fig 2), whereas *H. cachimbo* and *H. anataliasiasi* often have this limit above the tympanum (pattern D1), a pattern common to the three species. The presence of a thin white to pinkish stripe on the edges of the tibia above a thin brown stripe (pattern E1; fig 2) in many specimens of *H. rubicundula* distinguishes them from *H. cachimbo*, which never has such a pattern; also, no specimen of *H. rubicundula* has a thin longitudinal central brown stripe on the upper surface of tibia composed of thin dots (pattern E4), whereas many individuals of *H. anataliasiasi* have such a pattern. The presence in *H. rubicundula* of a thin pinkish to white canthal stripe above a brown loreal stripe (pattern F1; fig. 2) distinguishes it from *H cachimbo* which lacks such a pattern; also, the presence in many specimens of the former of a broad canthal pinkish stripe above a brown loreal stripe (patterns F2-F3) distinguishes them from *H. anataliasiasi*, which never has such a pattern. *Hyla rubicundula* has a truncate or rounded snout (fig. 6B-C, 7B-C), whereas *H. cachimbo* has an acuminate snout (fig. 6A, 7A), also, the former has a head as long as wide, its width being contained about 3.3 times in the snout-vent length, and *H anataliasiasi* has a head longer than wide, its width being contained about 3 6 times in the snout-vent length.

Description The following description is based on topotypes and other geographic samples from Minas Gerais and Bahia (morphospecies RU). The morphotype located in central Brazil (morphospecies PRU) is characterized in the geographic variation section.

Descriptive statistics are provided in tab. 5 Head as long as wide (n – 140, t – 1.65, P – 0 09), its width contained about 3.3 times in snout-vent length; internarial distance greater than eye-nostril distance (n – 139, t = 4.61, P = 0) and much smaller than eye diameter (n = 139, t = 50.29, P = 0); eye diameter greater than eye nostril distance (n = 139, t = 53 66, P = 0); canthus rostralis distinct, slightly rounded; lorus slightly oblique, sometimes perpendicular to canthus rostralis; eyes slightly to very prominent, tympanum distinct and nearly circular; supratympanic fold poorly developed; nostrils dorsolateral, slightly protuberant, directed laterally or slightly forward, internarial region furrowed or not, vomerine teeth in two patches between choanae, with irregular shape and position, tongue cordiform or rounded, vocal sac single and subgular.

Forearm more robust and shorter than upper arm (n – 139, t = 40.64, P – 0), hands with a distinct palmar tubercle, subarticular tubercles rounded, distal tubercle of fourth finger bifid, that of third finger bifid or rounded, supernumerary tubercles present, prepollex

Table 5 - Descriptive statistical tables of morphometric characters for *Hyla rubicundula* (morphospecies RU and PRU). n = number of specimens for which data are available, x = mean; s = standard deviation; CV = coefficient of variation

	Morphospecies RU											Morphospecies PRU										
Characters	Males						Females (n = 4)					Males						Females (n = 6)				
	n	*x*	*min*	*max*	*s*	*CV*	*x*	*min*	*max*	*s*	*CV*	*n*	*x*	*min*	*max*	*s*	*CV*	*x*	*min*	*max*	*s*	*CV*
SVL	140	21.27	18.0	23.4	0.97	4.58	23.75	21.6	25.1	1.52	6.43	47	21.67	18.1	23.8	1.09	5.07	23.93	22.2	25.4	1.43	5.98
HW	140	6.31	5.4	7.0	0.28	4.54	6.57	6.2	6.9	0.33	5.35	47	6.49	5.6	7.2	0.31	4.86	7.00	6.5	4.2	0.26	3.80
HL	140	6.37	5.5	7.1	0.27	4.36	6.81	6.5	7.1	0.33	4.88	47	6.45	5.7	7.0	0.26	4.15	7.05	6.5	7.4	0.30	4.37
ED	139	2.33	2.0	2.7	0.14	6.36	2.51	2.3	2.6	0.14	5.94	47	2.45	2.1	2.8	0.14	5.85	2.58	2.3	2.7	0.16	6.20
UEW	136	1.56	1.2	2.0	0.15	9.66	1.57	1.5	1.7	0.11	7.55	46	1.56	1.0	1.8	0.14	9.35	1.70	1.4	1.9	0.16	9.51
IOD	129	2.16	1.7	2.6	0.19	9.12	2.36	2.0	2.6	0.27	11.51	46	2.18	1.8	2.5	0.14	6.82	2.27	2.2	2.4	0.09	4.34
END	139	1.48	1.1	1.8	0.10	6.85	1.58	1.5	1.7	0.08	5.37	47	1.53	1.3	1.7	0.10	6.70	1.60	1.5	1.7	0.09	5.70
IND	139	1.55	1.1	1.8	0.10	6.85	1.58	1.5	1.7	0.11	6.98	47	1.54	1.3	1.8	0.09	6.08	1.70	1.5	1.8	0.08	5.26
THL	137	9.81	8.0	12.1	0.56	5.77	10.61	9.4	11.1	0.78	7.37	47	10.08	8.5	11.3	0.60	6.08	11.15	10.3	11.8	0.51	4.66
TL	140	9.99	8.3	11.1	0.48	4.88	10.76	9.7	11.3	0.73	6.85	47	10.05	8.3	11.1	0.58	5.85	11.04	10.2	11.5	0.59	5.36
TD	138	0.97	0.6	1.4	0.11	12.17	1.12	1.0	1.2	0.18	13.26	44	1.03	0.8	1.2	0.08	8.24	1.27	0.9	1.8	0.31	25.01
NSD	139	1.11	0.9	1.8	0.11	10.41	1.15	0.9	1.3	0.18	16.26	47	1.13	0.9	1.2	0.08	7.25	1.21	1.1	1.3	0.07	5.12
LAR	139	5.83	4.4	7.2	0.47	8.07	6.28	6.0	6.5	0.22	3.57	47	5.99	5.1	6.7	0.40	6.42	6.58	6.0	6.9	0.33	5.12
FAR	139	3.90	3.1	4.9	0.30	7.37	4.31	3.8	4.6	0.35	8.16	47	3.92	3.4	4.5	0.25	6.42	4.14	3.7	4.6	0.30	7.45
HAL	139	5.84	4.4	7.0	0.43	7.38	6.28	6.0	6.4	0.19	3.07	47	6.11	5.3	7.4	0.39	6.53	6.60	6.0	6.9	0.34	5.22
3FD	139	0.86	0.6	1.1	0.07	9.27	0.96	0.8	1.0	0.08	8.87	46	0.89	0.6	1.0	0.08	9.02	0.97	0.8	1.1	0.09	9.59
FL	139	14.61	11.7	16.3	0.76	5.26	15.72	14.3	16.5	0.96	6.14	47	14.89	12.2	17.4	14.89	1.00	16.20	15.0	17.4	1.01	6.26
4TD	139	0.80	0.5	1.0	0.09	11.68	0.87	0.8	0.9	0.06	7.37	47	0.84	0.6	1.0	0.09	11.57	0.88	0.7	1.0	0.10	11.69



Fig. 8. Hands and feet of adult males. (A) *Hyla cachimbo*, holotype, MZUSP 21912, Cachimbo, Para, (B) *H. rubicundula*, topotype, morphospecies RU, MNRJ 17294, Lagoa Santa, Minas Gerais; (C) *H. rubicundula*, morphospecies PRU, MNRJ 17295, Goiânia, Goiás, (D) *H. anataliasiasi*, MZUSP 49610, Posto Diauarum, Mato Grosso.

distinct, third finger disk diameter greater than fourth toe disk ($n = 139$, $t = 5.72$, $P = 0$); modal webbing formula, I 2.75-2.75 II 2-3.25 III 3-2.25 IV. Legs slender; femur and tibia with about the same stoutness; femur length shorter than tibia length ($n = 137$, $t = 2.88$, $P = 0$); sum of femur and tibia lengths smaller than snout-vent length ($n = 137$, $t = 12.20$, $P = 0$); toes not robust; subarticular tubercles rounded; supernumerary tubercles variable in shape and number; prehallux distinct, modal webbing formula, I 2-2.25 II 1^+-2.25 III 1^+-2.25 IV 2.25-1^+ V.

Color. In life, the analysis of four topotypic specimens from Lagoa Santa (Minas Gerais) revealed that in the same specimen the dorsal surfaces vary from dark green to dark brown, with an intermediate yellow phase, dots and dark brown stripes are not visible on the dorsum; a dark brown stripe, bordered by a white stripe, is visible on the flanks and canthus rostralis, thigh light brown and immaculate, vocal sac yellowish, belly white; finger and toe disks reddish.

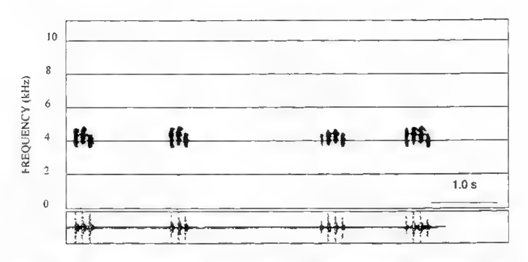


Fig 9. Sonogram and oscillogram of advertisement call of *Hyla rubicundula* (morphospecies PRU) from Silvânia, Goiás. Calls are given sporadically. The intervals between the notes are not natural

In preservative, dorsum reddish, with occasional dark brown stripes and dots (patterns A1-A10; fig 1); a mid-dorsal pin-stripe sometimes present on dorsum (patterns B1-B2; fig. 1); canthus rostralis delimited by a dark subcanthal brown stripe bordered above by a light pink to white stripe (pattern F1; fig 2); lorus with a variable degree of melanization; dorsolateral region delimited by a dark brown stripe bordered above or not by a light pink to white stripe from posterior corner of orbit to near groin (patterns C1-C2 and C5; fig. 2), both often above tympanum (pattern D1; fig. 2); thigh light brown, immaculate; a brown stripe sometimes present on anterior and posterior edges of tibia in addition to random dots (patterns E1-E3; fig. 2); ventral surfaces immaculate buff.

Geographic variation Samples from central Brazil (morphospecies PRU) have the following differences when compared to samples from Minas Gerais and Bahia (morphospecies RU). dorsal head shape pattern with pattern A7 (fig. 3, 6C, 7A); internarial distance and eye-nostril distance nearly equal ($n = 47$, $t = 0.26$, $P = 0.79$); lorus slightly to strongly concave, tympanum covered or not by a supratympanic fold, distal tubercle of fourth finger bifid or not, femur and tibia the same length ($n = 47$, $t = 0.22$, $P = 0.82$); dorsolateral stripes pattern corresponding to patterns C3-C4 (fig 2); lateral limits of dorsal pattern corresponding to pattern D2 (fig. 2). The other variations are of a matter of degree (tab. 1) and descriptive statistics are presented in tab. 5

Vocalization The advertisement calls studied are from one specimen from Silvânia, Goiás (morphospecies PRU; fig 9) Each note composed of three pulses had a duration of nearly 0 03 s, and each note was composed of four pulses about 0.04 s. Broadcast frequencies range between 3.5 and 4 8 kHz Air temperature was 21 5°C. CARDOSO & VIELLIARD (1985) gave a

detailed description of the call of *Hyla rubicundula* from Lagoa Santa, the type-locality of morphospecies RU. Comparisons between the two vocalizations reveal that they are very similar and that both belong to *H. rubicundula*.

Geographic distribution. *Hyla rubicundula* occurs in Minas Gerais, Goiás, Bahia and Piauí (fig. 4), mainly in the Cerrado Domain (Ab' Saber, 1977), and never crosses tropical rain forests.

Hyla anataliasiasi Bokermann, 1972

(fig. 6D, 7D, 8D)

Specimens examined. – BRAZIL. Mato Grosso: Posto Diauarum (MZUSP 49588-49617), Posto Leonardo (MZUSP 49339-49393).

Holotype. - WCAB 45272, adult male, collected at Belém-Brasília highroad, 80 km before Paraíso do Norte, Brejinho de Nazaré (about 11°00'S, 48°33'W), Goiás [Tocantins], Brazil, 247 m, 17 January 1970, by C. A Bokermann, Ladislau A. Deutsch and Milton S. Carollo.

Paratypes. Four adult males: WCAB 45273, collected with the holotype; WCAB 45256-45258, collected at Paranã (about 12°36'S, 47°52'W), Goiás [Tocantins], Brazil, 274 m, December 1969, by Anatalias J. Rodrigues.

Diagnosis. – Species characterized by the following combination of traits: (1) small size (SVL: males 16.0-21.8 mm; females 16.6-21.6 mm); (2) dorsum with nearly parallel dark brown stripes, the two anterior ones very near each other, joined with the two sacral ones (pattern A11; fig. 1); and (3) head longer than wide, its width being contained about 3.6 times in snout-vent length (fig 6D, 7D).

The presence of two anterior dorsal brown stripes fused to the sacral ones in some specimens of *H anataliasiasi* (pattern A11, fig. 1) distinguishes them from *H. rubicundula* and *H cachimbo*, which lack such a pattern; also, the absence in the former of two divergent dorsal brown stripes, from the anterior section of head to nearly half of the dorsum, barely separated from two sacral brown stripes (patterns A1 and A4), with or without additional dorsolateral stripes (patterns A5 and A8-A10), distinguishes it from *H rubicundula*, which has many individuals with such patterns. A mid-dorsal pin stripe (patterns B1 and B6, fig. 1) in many specimens of *H anataliasiasi* distinguishes them from *H cachimbo* in which stripes are absent A well-marked dark brown to black dorsolateral stripe under a thin white stripe (pattern C7; fig 2) in some specimens of *H anataliasiasi* distinguishes them from *H rubicundula* and *H cachimbo* which never possess such a pattern; also, the absence in the former of a broad and irregular brown dorsolateral stripe, with or without an upper white to pinkish stripe (patterns C3-C4), distinguishes it from many individuals of *H rubicundula* with such patterns. No specimen of *H cachimbo* has the lateral limits of the dorsal coloration below the lower border of the tympanum (pattern D2; fig. 2), but many individuals of *H rubicundula* from Goiás have such a pattern. The presence in some specimens of *H. anataliasiasi* of a thin white to pinkish stripe on the edges of tibia, above a thin brown stripe (pattern

E1; fig. 2), distinguishes them from *H. cachimbo*, which never has such a pattern; also, the presence in the former of a thin longitudinal central brown stripe on the upper surface of tibia, composed of small dots (pattern E4), distinguishes it from *H. rubicundula* and *H. cachimbo* which never possess such a pattern. No specimen of *H. anataliasiasi* has a broad canthal pinkish stripe above a brown loreal stripe (patterns F2-F3; fig. 2), but many individuals of *H. rubicundula* and *H. cachimbo* have such a pattern. The snout in *H. anataliasiasi* is acuminate in many individuals (fig 6D, 7D), but it is rounded or truncate in *H. rubicundula* (fig 6B-C, 7B-C). In the former the head is longer than wide, its width being contained about 3.6 times in snout-vent length, whereas in *H. rubicundula* and *H. cachimbo* the head is as long as wide, its width being contained, respectively, about 3.3 and 3.1 times in snout-vent length

Description. – Descriptive statistics are provided in tab. 4. Head longer than wide (n = 80, t = 6.23, P = 0), its width being contained about 3.6 times in snout-vent length; internarial distance greater than eye-nostril distance (n = 80, t = 3.09, P = 0) and much smaller than eye diameter (n = 80, t = 54.51, P = 0); eye diameter greater than eye-nostril distance (n = 80, t = 56.35, P = 0); snout truncate, rounded or acuminate in dorsal outline, and slightly protruding, truncate or rounded in lateral outline; canthus rostralis distinct, especially when bordered by loreal and canthal stripes, rounded or straight; lorus slightly concave; eyes moderately prominent, tympanum distinct, nearly circular; a supratympanic fold sometimes covering upper surface of tympanum; nostrils dorsolateral, slightly protuberant, directed laterally or slightly anteriorly; internarial region furrowed, vomerine teeth in two patches with irregular shapes and positions between choanae; tongue cordiform or rounded; vocal sac single, subgular, not well developed.

Forearm shorter and more robust than upper arm (n – 80, t = 33.04, P = 0), hands with a distinct palmar tubercle; subarticular tubercles distinct, rounded; distal tubercle of third and fourth fingers bifid or not; supernumerary tubercles present, palmar tubercle distinct; prepollex distinct, third finger disk diameter greater than fourth toe disk (n = 74, t = 4.92, P = 0); modal webbing formula, I 2.50-2.75 II 2.25-3.25 III 2.75-2.25 IV. Legs slender; femur and tibia with the same stoutness, femur longer than tibia (n = 80, t = 3.60, P = 0); sum of femur and tibia lengths smaller than snout-vent length (n = 80, t – 8.57, P = 0), foot with rounded subarticular tubercles, supernumerary tubercles not very distinct; prehallux distinct, plantar tubercle present or not, modal webbing formula, I 1.75-2.25 II 1^+-2.25 III 1.25-2 25 IV 3 -1^+ V.

Color In life, dorsal surfaces green (BOKERMANN, 1972). In preservative, dorsum reddish with occasional dark brown stripes and dots (patterns A2, A6 and A11; fig. 1); a mid-dorsal pin-stripe present or not (patterns B1-B2, fig 2); canthus rostralis delimited, or not, by a subcanthal dark brown stripe bordered above by a light pink to white stripe (patterns F1-F3, fig 2), lorus with a variable degree of melanization, a lateral brown stripe sometimes present on flanks from posterior corner of orbit to near groin, sometimes bordered by a light pinkish stripe (patterns C1-C2, C5 and C7; fig 2), both often above tympanum (pattern B1; fig. 2); thigh light brown with numerous widespread light brown dots; a brown stripe sometimes present on anterior and posterior edges of upper surface of tibia, bordered by a light pink to white stripe, in a addition to dorsal random dots (patterns E1 and E3; fig 2), or with a thin longitudinal central stripe composed of small dots (pattern E4), ventral surfaces immaculate buff

Geographic distribution. Recorded from Tocantins (Brejinho do Nazaré and Paranã; BOKERMANN, 1972) and northern Mato Grosso (Posto Diauarum and Posto Leonardo; fig. 4), both in the Cerrado Domain (AB' SABER, 1977) at elevations between 247 and 274 m

RÉSUMÉ

Le groupe d'espèces de *Hyla rubicundula*, composé de *H rubicundula* Reinhardt & Lütken, 1862 et *H. anataliasiasi* Bokermann, 1972, est subdivisé en quatre morpho-espèces. La variation intra- et inter-populationnelle de la morphologie externe de chaque morpho-espèce est analysée. *Hyla rubicundula* renferme trois des quatre morpho-espèces. Celle située au nord de sa répartition est décrite comme une espèce nouvelle, caractérisée principalement par un dos immaculé et un museau pointu. Une redescription est présentée pour les espèces *H rubicundula* et *H. anataliasiasi*.

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APPENDIX 1

Criteria for combination of patterns
in analyses of interpopulation variation
of morphospecies RU and PRU

Patterns were joined by similarity and geographic distribution

General dorsal patterns A1 and A4 are typical from topotypic samples for *Hyla rubicundula*. Compared to patterns A1 and A4, A2, A3, A6 and A7 are incomplete, vestigial or absent, whereas A5, A8, A9 and A10 have additional melanization.

Mid-dorsal pin stripe patterns. – B1 and B2, presence, B3, absence.

Dorsolateral stripes. – C1 and C2, typical from Lagoa Santa, Minas Gerais; C3 and C4, typical from Goiás; C5 and C6, vestigial or absent; C7, only for *H anataliasiasi*.

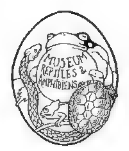
Dorsal head shape patterns. – G1-G3, typical from Lagoa Santa, Minas Gerais; G4-G5, typical from Barreiras, Bahia, G6-G7, typical from central Minas Gerais.

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Le corps de l'ouvrage présente les genres, espèces et sous-espèces de l'ordre des Chéloniens, classés par familles et sous-familles. Au sein de chaque catégorie, à partir du niveau de la super-famille, les taxons de rang immédiatement inférieur sont disposés selon l'ordre alphabétique, à l'exception du taxon-type d'une catégorie, quelle qu'elle soit, qui est toujours cité en premier. Ainsi, dans chaque genre, l'espèce-type est toujours placée en tête, et, au sein d'une espèce polytypique, il en est de même pour la sous-espèce nominative. En regard de chaque taxon sont indiqués d'abord le nom du descripteur suivi de l'année de sa publication, puis les noms vernaculaires français et anglais, s'ils sont connus, et enfin plusieurs (rarement une seule) références bibliographiques.

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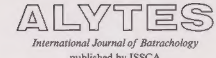
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